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(54) Title: POLYNUCLEOTIDES ENCODING ANTIGENIC HIV TYPE C POLYPEPTIDES, POLYPEPTIDES AND USES THEREOF

(57) Abstract: The present invention relates to polynucleotides encoding immunogenic HIV polypeptides. Uses of the polynucleotides in applications including immunization, generation of packaging cell lines, and production of HIV polypeptides are also described. Polynucleotides encoding antigenic HIV polypeptides are described, as are uses of these polynucleotides and polypeptide products therefrom, including formulations of immunogenic compositions and uses thereof.

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POLYNUCLEOTIDES ENCODING ANTIGENIC HIV TYPE C POLYPEPTIDES, POLYPEPTIDES AND USES THEREOF

TECHNICAL FIELD

Polynucleotides encoding antigenic HIV polypeptides (*e.g.*, those shown in
5 Table C) are described, as are uses of these polynucleotides and polypeptide products
including formulations of immunogenic compositions and uses thereof.

BACKGROUND OF THE INVENTION

Acquired immune deficiency syndrome (AIDS) is recognized as one of the
10 greatest health threats facing modern medicine. There is, as yet, no cure for this
disease.

In 1983-1984, three groups independently identified the suspected etiological
agent of AIDS. See, *e.g.*, Barre-Sinoussi et al. (1983) *Science* 220:868-871;
Montagnier et al., in *Human T-Cell Leukemia Viruses* (Gallo, Essex & Gross, eds.,
15 1984); Vilmer et al. (1984) *The Lancet* 1:753; Popovic et al. (1984) *Science*
224:497-500; Levy et al. (1984) *Science* 225:840-842. These isolates were variously
called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus
type III (HTLV-III), or AIDS-associated retrovirus (ARV). All of these isolates are
strains of the same virus, and were later collectively named Human Immunodeficiency
20 Virus (HIV). With the isolation of a related AIDS-causing virus, the strains originally
called HIV are now termed HIV-1 and the related virus is called HIV-2 See, *e.g.*,
Guyader et al. (1987) *Nature* 326:662-669; Brun-Vezinet et al. (1986) *Science*
233:343-346; Clavel et al. (1986) *Nature* 324:691-695.

A great deal of information has been gathered about the HIV virus, however,
25 to date an effective vaccine has not been identified. Several targets for vaccine
development have been examined including the *env* and *Gag* gene products encoded
by HIV. *Gag* gene products include, but are not limited to, *Gag*-polymerase and *Gag*-
protease. *Env* gene products include, but are not limited to, monomeric gp120
polypeptides, oligomeric gp140 polypeptides and gp160 polypeptides.

30 Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective
codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency

of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with modified codon usage. Schneider, et al., (*J Virol.* 71(7):4892-4903, 1997) discuss inactivation of inhibitory (or instability) elements (INS) located
5 within the coding sequences of the Gag and Gag-protease coding sequences.

The *Gag* proteins of HIV-1 are necessary for the assembly of virus-like particles. HIV-1 *Gag* proteins are involved in many stages of the life cycle of the virus including, assembly, virion maturation after particle release, and early post-entry steps in virus replication. The roles of HIV-1 *Gag* proteins are numerous and complex
10 (Freed, E.O., *Virology* 251:1-15, 1998).

Wolf, et al., (PCT International Application, WO 96/30523, published 3 October 1996; European Patent Application, Publication No. 0 449 116 A1, published 2 October 1991) have described the use of altered pr55 *Gag* of HIV-1 to act as a non-infectious retroviral-like particulate carrier, in particular, for the presentation of
15 immunologically important epitopes. Wang, et al., (*Virology* 200:524-534, 1994) describe a system to study assembly of HIV Gag- β -galactosidase fusion proteins into virions. They describe the construction of sequences encoding HIV Gag- β -galactosidase fusion proteins, the expression of such sequences in the presence of HIV *Gag* proteins, and assembly of these proteins into virus particles.

20 Shiver, et al., (PCT International Application, WO 98/34640, published 13 August 1998) described altering HIV-1 (CAM1) *Gag* coding sequences to produce synthetic DNA molecules encoding HIV *Gag* and modifications of HIV *Gag*. The codons of the synthetic molecules were codons preferred by a projected host cell.

Recently, use of HIV Env polypeptides in immunogenic compositions has been
25 described. (see, U.S. Patent No. 5,846,546 to Hurwitz et al., issued December 8, 1998, describing immunogenic compositions comprising a mixture of at least four different recombinant virus that each express a different HIV env variant; and U.S. Patent No. 5,840,313 to Vahlne et al., issued November 24, 1998, describing peptides which correspond to epitopes of the HIV-1 gp120 protein). In addition, U.S. Patent
30 No. 5,876,731 to Sia et al, issued March 2, 1999 describes candidate vaccines against HIV comprising an amino acid sequence of a T-cell epitope of Gag linked directly to

an amino acid sequence of a B-cell epitope of the V3 loop protein of an HIV-1 isolate containing the sequence GPGR.

SUMMARY OF THE INVENTION

- 5 Described herein are novel HIV sequences, polypeptides encoded by these novel sequences, and synthetic expression cassettes generated from these and other HIV sequences. In one aspect, the present invention relates to improved HIV expression cassettes. In a second aspect, the present invention relates to generating an immune response in a subject using the expression cassettes of the present invention.
- 10 In a further aspect, the present invention relates to generating an immune response in a subject using the expression cassettes of the present invention, as well as, polypeptides encoded by the expression cassettes of the present invention. In another aspect, the present invention relates to enhanced vaccine technologies for the induction of potent neutralizing antibodies and/or cellular immune responses against HIV in a subject.
- 15 In certain embodiments, the present invention relates to isolated wild-type polynucleotides and/or expression cassettes encoding HIV polypeptides, including, but not limited to, Env, Gag, Pol, Prot, RT, Int, Vpr, Vpu, Vif, Nef, Tat, Rev and/or combinations and fragments thereof. Mutations in some of the genes are described that reduce or eliminate the activity of the gene product without adversely affecting the
- 20 ability of the gene product to generate an immune response. Exemplary polynucleotides include, but are not limited to, *EnvTV001c8.2* (SEQ ID NO:61), *EnvTV001c8.5* (SEQ ID NO:62), *EnvTV001c12.1* (SEQ ID NO:63), *EnvTV003cE260* (SEQ ID NO:64), *EnvTV004cC300* (SEQ ID NO:65), *EnvTV006c9.1* (SEQ ID NO:66), *EnvTV006c9.2* (SEQ ID NO:67), *EnvTV006cE9* (SEQ ID NO:68),
- 25 *EnvTV007cB104* (SEQ ID NO:69), *EnvTV007cB105* (SEQ ID NO:70), *EnvTV008c4.3* (SEQ ID NO:71), *EnvTV008c4.4* (SEQ ID NO:72), *EnvTV010cD7* (SEQ ID NO:73), *EnvTV012c2.1* (SEQ ID NO:74), *EnvTV012c2.2* (SEQ ID NO:75), *EnvTV013cB20* (SEQ ID NO:76), *EnvTV013cH17* (SEQ ID NO:77), *EnvTV014c6.3* (SEQ ID NO:78), *EnvTV014c6.4* (SEQ ID NO:79),
- 30 *EnvTV018cF1027* (SEQ ID NO:80), *EnvTV019c5* (SEQ ID NO:81), *GagTV001G8* (SEQ ID NO:82), *GagTV001G11* (SEQ ID NO:83), *GagTV002G8* (SEQ ID NO:84),

5 *GagTV003G15* (SEQ ID NO:85), *GagTV004G17* (SEQ ID NO:86), *GagTV004G24* (SEQ ID NO:87), *GagTV006G11* (SEQ ID NO:88), *GagTV006G97* (SEQ ID NO:89), *GagTV007G59* (SEQ ID NO:90), *GagTV008G65* (SEQ ID NO:91), *GagTV008G66* (SEQ ID NO:92), *GagTV010G74* (SEQ ID NO:93), *GagTV012G34* (SEQ ID NO:94), *GagTV012G40* (SEQ ID NO:95), *GagTV013G2* (SEQ ID NO:96), *GagTV013G15* (SEQ ID NO:97), *GagTV014G73* (SEQ ID NO:98), *GagTV018G60* (SEQ ID NO:99), *GagTV019G20* (SEQ ID NO:100), *GagTV019G25* (SEQ ID NO:101), 8_2_TV1 LTR (SEQ ID NO:181), and 2_1/4_TV12_C_ZA (SEQ ID NO:182).

10 In other embodiments, the present invention relates synthetic polynucleotides and/or expression cassettes encoding HIV polypeptides, including but not limited to Env, Gag, Pol, Prot, Int, Vpr, Vpu, Vif, Nef, Tat, Rev and/or combinations and fragments thereof. In addition, the present invention also relates to improved expression of HIV polypeptides and production of virus-like particles. Synthetic
 15 expression cassettes encoding the HIV polypeptides (*e.g.*, Gag-, pol-, protease (prot)-, reverse transcriptase, integrase, RNaseH, Tat, Rev, Nef, Vpr, Vpu, Vif and/or Env-containing polypeptides) are described, as are uses of the expression cassettes. Mutations in some of the genes are described that reduce or eliminate the activity of the gene product without adversely affecting the ability of the gene product to
 20 generate an immune response. Exemplary synthetic polynucleotides include, but are not limited to, *GagComplPolmut_C* (SEQ ID NO:9), *GagComplPolmutAtt_C* (SEQ ID NO:10), *GagComplPolmutIna_C* (SEQ ID NO:11), *GagComplPolmutInaTatRevNef_C* (SEQ ID NO:12), *GagPolmut_C* (SEQ ID NO:13), *GagPolmutAtt_C* (SEQ ID NO:14), *GagPolmutIna_C* (SEQ ID NO:15),
 25 *GagProtInaRTmut_C* (SEQ ID NO:16), *GagProtInaRTmutTatRevNef_C* (SEQ ID NO:17), *GagRTmut_C* (SEQ ID NO:18), *GagRTmutTatRevNef_C* (SEQ ID NO:19), *GagTatRevNef_C* (SEQ ID NO:20), *gp120mod.TV1.del118-210* (SEQ ID NO:21), *gp120mod.TV1.delV1V2* (SEQ ID NO:22), *gp120mod.TV1.delV2* (SEQ ID NO:23), *gp140mod.TV1.del118-210* (SEQ ID NO:24), *gp140mod.TV1.delV1V2* (SEQ ID
 30 NO:25), *gp140mod.TV1.delV2* (SEQ ID NO:26); *gp140mod.TV1.mut7* (SEQ ID NO:27), *gp140mod.TV1.tpa2* (SEQ ID NO:28), *gp140TMmod.TV1* (SEQ ID

NO:29), gp160mod.TV1.del118-210 (SEQ ID NO:30), gp160mod.TV1.delV1V2 (SEQ ID NO:31), gp160mod.TV1.delV2 (SEQ ID NO:32), gp160mod.TV1.dV1 (SEQ ID NO:33), gp160mod.TV1.dV1-gagmod.BW965 (SEQ ID NO:34), gp160mod.TV1.dV1V2-gagmod.BW965 (SEQ ID NO:35), gp160mod.TV1.dV2-gagmod.BW965 (SEQ ID NO:36), gp160mod.TV1.tpa2 (SEQ ID NO:37), gp160mod.TV1-gagmod.BW965 (SEQ ID NO:38), int.opt.mut_C (SEQ ID NO:39), int.opt_C (SEQ ID NO:40), nef.D106G.-myr19.opt_C (SEQ ID NO:41), p15RnaseH.opt_C (SEQ ID NO:42), p2Pol.opt.YMWM_C (SEQ ID NO:43), p2Polopt.YM_C (SEQ ID NO:44), p2Polopt_C (SEQ ID NO:45), p2PolTatRevNef opt C (SEQ ID NO:46), p2PolTatRevNef.opt.native_C (SEQ ID NO:47), p2PolTatRevNef.opt_C (SEQ ID NO:48), protInaRT.YM.opt_C (SEQ ID NO:49), protInaRT.YMWM.opt_C (SEQ ID NO:50), ProtRT.TatRevNef.opt_C (SEQ ID NO:51), rev.exon1_2.M5-10.opt_C (SEQ ID NO:52), tat.exon1_2.opt.C22-37_C (SEQ ID NO:53), tat.exon1_2.opt.C37_C (SEQ ID NO:54), TatRevNef.opt.native_ZA (SEQ ID NO:55), TatRevNef.opt_ZA (SEQ ID NO:56), TatRevNefGag C (SEQ ID NO:57), TatRevNefgagCpollna C (SEQ ID NO:58), TatRevNefGagProtInaRTmut C (SEQ ID NO:59), TatRevNefProtRT opt C (SEQ ID NO:60), gp140.modTV1.mut1.dV2 (SEQ ID NO:183); gp140mod.TV1.mut2.dV2 (SEQ ID NO:184), gp140mod.TV1.mut3.dV2 (SEQ ID NO:185), gp140mod.TV1.mut4.dV2 (SEQ ID NO:186), gp140.mod.TV1.GM161 (SEQ ID NO:187), gp140mod.TV1.GM161-195-204 (SEQ ID NO:188), gp140mod.TV1.GM161-204 (SEQ ID NO:189), gp140mod.TV1.GM-V1V2 (SEQ ID NO:190), gp140modC8.2mut7.delV2.Kozmod.Ta (SEQ ID NO:191), and Nef-myrD124LLAA (SEQ ID NO:203).

Thus, one aspect of the present invention relates to expression cassettes and polynucleotides contained therein. The expression cassettes typically include an HIV-polypeptide encoding sequence inserted into an expression vector backbone. In one embodiment, an expression cassette comprises a polynucleotide sequence encoding one or more polypeptides, wherein the polynucleotide sequence comprises a sequence having between about 85% to 100% and any integer values therebetween, for example, at least about 85%, preferably about 90%, more preferably about 95%, and more

preferably about 98% sequence identity to the sequences taught in the present specification.

The polynucleotides encoding the HIV polypeptides of the present invention may also include sequences encoding additional polypeptides. Such additional
 5 polynucleotides encoding polypeptides may include, for example, coding sequences for other viral proteins (*e.g.*, hepatitis B or C or other HIV proteins, such as, polynucleotide sequences encoding an HIV *Gag* polypeptide, polynucleotide sequences encoding an HIV *Env* polypeptide and/or polynucleotides encoding one or more of *vif*, *vpr*, *tat*, *rev*, *vpu* and *nef*); cytokines or other transgenes.

10 In one embodiment, the sequence encoding the HIV *Pol* polypeptide(s) can be modified by deletions of coding regions corresponding to reverse transcriptase and integrase. Such deletions in the polymerase polypeptide can also be made such that the polynucleotide sequence preserves T-helper cell and CTL epitopes. Other antigens of interest may be inserted into the polymerase as well.

15 In another embodiment, an expression cassette comprises a polynucleotide sequence encoding a polypeptide, for example, GagComplPolmut_C (SEQ ID NO:9), GagComplPolmutAtt_C (SEQ ID NO:10), GagComplPolmutIna_C (SEQ ID NO:11), GagComplPolmutInaTatRevNef_C (SEQ ID NO:12), GagPolmut_C (SEQ ID NO:13), GagPolmutAtt_C (SEQ ID NO:14), GagPolmutIna_C (SEQ ID NO:15),
 20 GagProtInaRTmut_C (SEQ ID NO:16), GagProtInaRTmutTatRevNef_C (SEQ ID NO:17), GagRTmut_C (SEQ ID NO:18), GagRTmutTatRevNef_C (SEQ ID NO:19), GagTatRevNef_C (SEQ ID NO:20), gp120mod.TV1.del118-210 (SEQ ID NO:21), gp120mod.TV1.delV1V2 (SEQ ID NO:22), gp120mod.TV1.delV2 (SEQ ID NO:23), gp140mod.TV1.del118-210 (SEQ ID NO:24), gp140mod.TV1.delV1V2 (SEQ ID
 25 NO:25), gp140mod.TV1.delV2 (SEQ ID NO:26), gp140mod.TV1.mut7 (SEQ ID NO:27), gp140mod.TV1.tpa2 (SEQ ID NO:28), gp140TMmod.TV1 (SEQ ID NO:29), gp160mod.TV1.del118-210 (SEQ ID NO:30), gp160mod.TV1.delV1V2 (SEQ ID NO:31), gp160mod.TV1.delV2 (SEQ ID NO:32), gp160mod.TV1.dV1 (SEQ ID NO:33), gp160mod.TV1.dV1-gagmod.BW965 (SEQ ID NO:34),
 30 gp160mod.TV1.dV1V2-gagmod.BW965 (SEQ ID NO:35), gp160mod.TV1.dV2-gagmod.BW965 (SEQ ID NO:36), gp160mod.TV1.tpa2 (SEQ ID NO:37),

gp160mod.TV1-gagmod.BW965 (SEQ ID NO:38), int.opt.mut_C (SEQ ID NO:39),
 int.opt_C (SEQ ID NO:40), nef.D106G.-myr19.opt_C (SEQ ID NO:41),
 p15RnaseH.opt_C (SEQ ID NO:42), p2Pol.opt.YMWM_C (SEQ ID NO:43),
 p2Polopt.YM_C (SEQ ID NO:44), p2Polopt_C (SEQ ID NO:45), p2PolTatRevNef
 5 opt C (SEQ ID NO:46), p2PolTatRevNef.opt.native_C (SEQ ID NO:47),
 p2PolTatRevNef.opt_C (SEQ ID NO:48), protInaRT.YM.opt_C (SEQ ID NO:49),
 protInaRT.YMWM.opt_C (SEQ ID NO:50), ProtRT.TatRevNef.opt_C (SEQ ID
 NO:51), rev.exon1_2.M5-10.opt_C (SEQ ID NO:52), tat.exon1_2.opt.C22-37_C
 (SEQ ID NO:53), tat.exon1_2.opt.C37_C (SEQ ID NO:54),
 10 TatRevNef.opt.native_ZA (SEQ ID NO:55), TatRevNef.opt_ZA (SEQ ID NO:56),
 TatRevNefGag C (SEQ ID NO:57), TatRevNefgagCpollna C (SEQ ID NO:58),
 TatRevNefGagProtInaRTmut C (SEQ ID NO:59), and TatRevNefProtRT opt C (SEQ
 ID NO:60), wherein the polynucleotide sequence encoding the polypeptide comprises
 a sequence having between about 85% to 100% and any integer values therebetween,
 15 for example, at least about 85%, preferably about 90%, more preferably about 95%,
 and more preferably about 98% sequence identity to the sequences taught in the
 present specification.

The native and synthetic polynucleotide sequences encoding the HIV
 polypeptides of the present invention typically have between about 85% to 100% and
 20 any integer values therebetween, for example, at least about 85%, preferably about
 90%, more preferably about 95%, and more preferably about 98% sequence identity to
 the sequences taught herein. Further, in certain embodiments, the polynucleotide
 sequences encoding the HIV polypeptides of the invention will exhibit 100% sequence
 identity to the sequences taught herein.

25 The polynucleotides of the present invention can be produced by recombinant
 techniques, synthetic techniques, or combinations thereof.

The present invention further includes recombinant expression systems for use
 in selected host cells, wherein the recombinant expression systems employ one or more
 of the polynucleotides and expression cassettes of the present invention. In such
 30 systems, the polynucleotide sequences are operably linked to control elements
 compatible with expression in the selected host cell. Numerous expression control

elements are known to those in the art, including, but not limited to, the following: transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences. Exemplary transcription promoters
5 include, but are not limited to those derived from CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

In another aspect the invention includes cells comprising one or more of the expression cassettes of the present invention where the polynucleotide sequences are operably linked to control elements compatible with expression in the selected cell. In
10 one embodiment such cells are mammalian cells. Exemplary mammalian cells include, but are not limited to, BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells. Other cells, cell types, tissue types, etc., that may be useful in the practice of the present invention include, but are not limited to, those obtained from the following: insects (e.g., *Trichoplusia ni* (Tn5) and Sf9), bacteria, yeast, plants, antigen presenting
15 cells (e.g., macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), primary cells, immortalized cells, tumor-derived cells.

In a further aspect, the present invention includes compositions for generating an immunological response, where the composition typically comprises at least one of the expression cassettes of the present invention and may, for example, contain
20 combinations of expression cassettes such as one or more expression cassettes carrying a Pol-derived-polypeptide-encoding polynucleotide, one or more expression cassettes carrying a Gag-derived-polypeptide-encoding polynucleotide, one or more expression cassettes carrying accessory polypeptide-encoding polynucleotides (e.g., native or synthetic vpu, vpr, nef, vif, tat, rev), and/or one or more expression cassettes carrying
25 an Env-derived-polypeptide-encoding polynucleotide. Such compositions may further contain an adjuvant or adjuvants. The compositions may also contain one or more HIV polypeptides. The HIV polypeptides may correspond to the polypeptides encoded by the expression cassette(s) in the composition, or may be different from those encoded by the expression cassettes. In compositions containing both
30 expression cassettes (or polynucleotides of the present invention) and polypeptides,

various expression cassettes of the present invention can be mixed and/or matched with various HIV polypeptides described herein.

In another aspect the present invention includes methods of immunization of a subject. In the method any of the above described compositions are into the subject
5 under conditions that are compatible with expression of the expression cassette(s) in the subject. In one embodiment, the expression cassettes (or polynucleotides of the present invention) can be introduced using a gene delivery vector. The gene delivery vector can, for example, be a non-viral vector or a viral vector. Exemplary viral vectors include, but are not limited to eucaryotic layered vector initiation systems,
10 Sindbis-virus (or other alphavirus) derived vectors, retroviral vectors, and lentiviral vectors. Other exemplary vectors include, but are not limited to, pCMVKm2, pCMV6a, pCMV-link, and pCMVPLEdhfr. Compositions useful for generating an immunological response can also be delivered using a particulate carrier (e.g., PLG or CTAB-PLG microparticles). Further, such compositions can be coated on, for
15 example, gold or tungsten particles and the coated particles delivered to the subject using, for example, a gene gun. The compositions can also be formulated as liposomes. In one embodiment of this method, the subject is a mammal and can, for example, be a human.

In a further aspect, the invention includes methods of generating an immune
20 response in a subject. Any of the expression cassettes described herein can be expressed in a suitable cell to provide for the expression of the HIV polypeptides encoded by the polynucleotides of the present invention. The polypeptide(s) are then isolated (e.g., substantially purified) and administered to the subject in an amount sufficient to elicit an immune response. In certain embodiments, the methods comprise
25 administration of one or more of the expression cassettes or polynucleotides of the present invention, using any of the gene delivery techniques described herein. In other embodiments, the methods comprise co-administration of one or more of the expression cassettes or polynucleotides of the present invention and one or more polypeptides, wherein the polypeptides can be expressed from these polynucleotides or
30 can be other HIV polypeptides. In other embodiments, the methods comprise co-administration of multiple expression cassettes or polynucleotides of the present

invention. In still further embodiments, the methods comprise co-administration of multiple polypeptides, for example polypeptides expressed from the polynucleotides of the present invention and/or other HIV polypeptides.

5 The invention further includes methods of generating an immune response in a subject, where cells of a subject are transfected with any of the above-described expression cassettes or polynucleotides of the present invention, under conditions that permit the expression of a selected polynucleotide and production of a polypeptide of interest (e.g., encoded by any expression cassette of the present invention). By this method an immunological response to the polypeptide is elicited in the subject.

10 Transfection of the cells may be performed *ex vivo* and the transfected cells are reintroduced into the subject. Alternately, or in addition, the cells may be transfected *in vivo* in the subject. The immune response may be humoral and/or cell-mediated (cellular). In a further embodiment, this method may also include administration of an HIV polypeptides before, concurrently with, and/or after introduction of the

15 expression cassette into the subject.

The polynucleotides of the present invention may be employed singly or in combination. The polynucleotides of the present invention, encoding HIV-derived polypeptides, may be expressed in a variety of ways, including, but not limited to the following: a polynucleotide encoding a single gene product (or portion thereof)

20 expressed from a promoter; multiple polynucleotides encoding a more than one gene product (or portion thereof) (e.g., polycistronic coding sequences); multiple polynucleotides in-frame to produce a single polypeptide; and, multiple polynucleotides in-frame to produce a single polypeptide wherein the polypeptide has protein cleavage sites between one or more of the polypeptides comprising the polypeptide.

25 These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A to 1D depict the nucleotide sequence of HIV Type C

30 8_5_TV1_C.ZA (SEQ ID NO:1; referred to herein as TV1). Various regions are shown in Table A.

Figures 2A-C depicts an alignment of Env polypeptides from various HIV isolates (SF162, SEQ ID NO:2; TV1.8_2, SEQ ID NO:3; TV1.8_5, SEQ ID NO:4; TV2.12-5/1, SEQ ID NO:5; Consensus Sequence, SEQ ID NO:6). The regions between the arrows indicate regions (of TV1 and TV2 clones, both HIV Type C isolates) in the beta and/or bridging sheet region(s) that can be deleted and/or truncated. The "*" denotes N-linked glycosylation sites (of TV1 and TV2 clones), one or more of which can be modified (*e.g.*, deleted and/or mutated).

Figure 3 presents a schematic diagram showing the relationships between the following forms of the HIV Env polypeptide: gp160, gp140, gp120, and gp41.

Figure 4 presents exemplary data concerning transactivation activity of Tat mutants on LTR-CAT plasmid expression in 293 cells.

Figure 5 presents exemplary data concerning export activity of Rev mutants monitored by CAT expression.

Figure 6, sheets 1 and 2, presents the sequence of the construct GagComplPolmut_C (SEQ ID NO:9).

Figure 7, sheets 1 and 2, presents the sequence of the construct GagComplPolmutAtt_C (SEQ ID NO:10).

Figure 8, sheets 1 and 2, presents the sequence of the construct GagComplPolmutIna_C (SEQ ID NO:11).

Figure 9, sheets 1 and 2, presents the sequence of the construct GagComplPolmutInaTatRevNef_C (SEQ ID NO:12).

Figure 10, presents the sequence of the construct GagPolmut_C (SEQ ID NO:13).

Figure 11, presents the sequence of the construct GagPolmutAtt_C (SEQ ID NO:14).

Figure 12, presents the sequence of the construct GagPolmutIna_C (SEQ ID NO:15).

Figure 13, presents the sequence of the construct GagProtInaRTmut_C (SEQ ID NO:16).

Figure 14, sheets 1 and 2, presents the sequence of the construct GagProtInaRTmutTatRevNef_C (SEQ ID NO:17).

Figure 15, presents the sequence of the construct GagRTmut_C (SEQ ID NO:18).

Figure 16, sheets 1 and 2, presents the sequence of the construct GagRTmutTatRevNef_C (SEQ ID NO:19).

5 Figure 17, presents the sequence of the construct GagTatRevNef_C (SEQ ID NO:20).

Figure 18, presents the sequence of the construct gp120mod.TV1.del118-210 (SEQ ID NO:21).

10 Figure 19, presents the sequence of the construct gp120mod.TV1.delV1V2 (SEQ ID NO:22).

Figure 20, presents the sequence of the construct gp120mod.TV1.delV2 (SEQ ID NO:23).

Figure 21, presents the sequence of the construct gp140mod.TV1.del118-210 (SEQ ID NO:24).

15 Figure 22, presents the sequence of the construct gp140mod.TV1.delV1V2 (SEQ ID NO:25).

Figure 23, presents the sequence of the construct gp140mod.TV1.delV2 (SEQ ID NO:26).

20 Figure 24, presents the sequence of the construct gp140mod.TV1.mut7 (SEQ ID NO:27).

Figure 25, presents the sequence of the construct gp140mod.TV1.tpa2 (SEQ ID NO:28).

Figure 26, presents the sequence of the construct gp140TMmod.TV1 (SEQ ID NO:29).

25 Figure 27, presents the sequence of the construct gp160mod.TV1.del118-210 (SEQ ID NO:30).

Figure 28, presents the sequence of the construct gp160mod.TV1.delV1V2 (SEQ ID NO:31).

30 Figure 29, presents the sequence of the construct gp160mod.TV1.delV2 (SEQ ID NO:32).

Figure 30, presents the sequence of the construct gp160mod.TV1.dV1 (SEQ ID NO:33).

Figure 31, sheets 1 and 2, presents the sequence of the construct gp160mod.TV1.dV1-gagmod.BW965 (SEQ ID NO:34).

5 Figure 32, sheets 1 and 2, presents the sequence of the construct gp160mod.TV1.dV1V2-gagmod.BW965 (SEQ ID NO:35).

Figure 33, sheets 1 and 2, presents the sequence of the construct gp160mod.TV1.dV2-gagmod.BW965 (SEQ ID NO:36).

10 Figure 34, presents the sequence of the construct gp160mod.TV1.tpa2 (SEQ ID NO:37).

Figure 35, sheets 1 and 2, presents the sequence of the construct gp160mod.TV1-gagmod.BW965 (SEQ ID NO:38).

Figure 36, presents the sequence of the construct int.opt.mut_C (SEQ ID NO:39).

15 Figure 37, presents the sequence of the construct int.opt_C (SEQ ID NO:40).

Figure 38, presents the sequence of the construct nef.D106G.-myr19.opt_C (SEQ ID NO:41).

Figure 39, presents the sequence of the construct p15RnaseH.opt_C (SEQ ID NO:42).

20 Figure 40, presents the sequence of the construct p2Pol.opt.YMWM_C (SEQ ID NO:43).

Figure 41, presents the sequence of the construct p2Polopt.YM_C (SEQ ID NO:44).

25 Figure 42, presents the sequence of the construct p2Polopt_C (SEQ ID NO:45).

Figure 43, presents the sequence of the construct p2PolTatRevNef.opt C (SEQ ID NO:46).

Figure 44, presents the sequence of the construct p2PolTatRevNef.opt.native_C (SEQ ID NO:47).

30 Figure 45, presents the sequence of the construct p2PolTatRevNef.opt_C (SEQ ID NO:48).

Figure 46, presents the sequence of the construct protInaRT.YM.opt_C (SEQ ID NO:49).

Figure 47, presents the sequence of the construct protInaRT.YMW.M.opt_C (SEQ ID NO:50).

5 Figure 48, presents the sequence of the construct ProtRT.TatRevNef.opt_C (SEQ ID NO:51).

Figure 49, presents the sequence of the construct rev.exon1_2.M5-10.opt_C (SEQ ID NO:52).

10 Figure 50, presents the sequence of the construct tat.exon1_2.opt.C22-37_C (SEQ ID NO:53).

Figure 51, presents the sequence of the construct tat.exon1_2.opt.C37_C (SEQ ID NO:54).

Figure 52, presents the sequence of the construct TatRevNef.opt.native_ZA (SEQ ID NO:55).

15 Figure 53, presents the sequence of the construct TatRevNef.opt_ZA (SEQ ID NO:56).

Figure 54, presents the sequence of the construct TatRevNefGag C (SEQ ID NO:57).

20 Figure 55, sheets 1 and 2, presents the sequence of the construct TatRevNefgagCpolIna C (SEQ ID NO:58).

Figure 56, sheets 1 and 2, presents the sequence of the construct TatRevNefGagProtInaRTmut C (SEQ ID NO:59).

Figure 57, presents the sequence of the construct TatRevNefProtRT opt C (SEQ ID NO:60).

25 Figure 58 presents the sequence of *Env* of clone TV001c8.2 of isolate C-98TV001 (SEQ ID NO:61).

Figure 59 presents the sequence of *Env* of clone TV001c8.5 of isolate C-98TV001 (SEQ ID NO:62).

30 Figure 60 presents the sequence of *Env* of clone TV001c12.1 of isolate C-98TV002 (SEQ ID NO:63).

Figure 61 presents the sequence of *Env* of clone TV003cE260 of isolate C-98TV003 (SEQ ID NO:64).

Figure 62 presents the sequence of *Env* of clone TV004cC300 of isolate C-98TV004 (SEQ ID NO:65).

5 Figure 63 presents the sequence of *Env* of clone TV006c9.1 of isolate C-98TV006 (SEQ ID NO:66).

Figure 64 presents the sequence of *Env* of clone TV006c9.2 of isolate C-98TV006 (SEQ ID NO:67).

10 Figure 65 presents the sequence of *Env* of clone TV006cE9 of isolate C-98TV006 (SEQ ID NO:68).

Figure 66 presents the sequence of *Env* of clone TV007cB104 of isolate C-98TV007 (SEQ ID NO:69).

Figure 67 presents the sequence of *Env* of clone TV007cB105 of isolate C-98TV007 (SEQ ID NO:70).

15 Figure 68 presents the sequence of *Env* of clone TV008c4.3 of isolate C-98TV008 (SEQ ID NO:71).

Figure 69 presents the sequence of *Env* of clone TV008c4.4 of isolate C-98TV008 (SEQ ID NO:72).

20 Figure 70 presents the sequence of *Env* of clone TV010cD7 of isolate C-98TV010 (SEQ ID NO:73).

Figure 71 presents the sequence of *Env* of clone TV012c2.1 of isolate C-98TV012 (SEQ ID NO:74).

Figure 72 presents the sequence of *Env* of clone TV012c2.2 of isolate C-98TV012 (SEQ ID NO:75).

25 Figure 73 presents the sequence of *Env* of clone TV013cB20 of isolate C-98TV013 (SEQ ID NO:76).

Figure 74 presents the sequence of *Env* of clone TV013cH17 of isolate C-98TV013 (SEQ ID NO:77).

30 Figure 75 presents the sequence of *Env* of clone TV014c6.3 of isolate C-98TV014 (SEQ ID NO:78).

Figure 76 presents the sequence of *Env* of clone TV014c6.4 of isolate C-98TV014 (SEQ ID NO:79).

Figure 77 presents the sequence of *Env* of clone TV018cF1027 of isolate C-98TV018 (SEQ ID NO:80).

5 Figure 78 presents the sequence of *Env* of clone TV019c5 of isolate C-98TV019 (SEQ ID NO:81).

Figure 79 presents the sequence of *Gag* of clone TV001G8 of isolate C-98TV001 (SEQ ID NO:82).

10 Figure 80 presents the sequence of *Gag* of clone TV001G11 of isolate C-98TV001 (SEQ ID NO:83).

Figure 81 presents the sequence of *Gag* of clone TV002G8 of isolate C-98TV002 (SEQ ID NO:84).

Figure 82 presents the sequence of *Gag* of clone TV003G15 of isolate C-98TV003 (SEQ ID NO:85).

15 Figure 83 presents the sequence of *Gag* of clone TV004G17 of isolate C-98TV004 (SEQ ID NO:86).

Figure 84 presents the sequence of *Gag* of clone TV004G24 of isolate C-98TV004 (SEQ ID NO:87).

20 Figure 85 presents the sequence of *Gag* of clone TV006G11 of isolate C-98TV006 (SEQ ID NO:88).

Figure 86 presents the sequence of *Gag* of clone TV006G97 of isolate C-98TV006 (SEQ ID NO:89).

Figure 87 presents the sequence of *Gag* of clone TV007G59 of isolate C-98TV009 (SEQ ID NO:90).

25 Figure 88 presents the sequence of *Gag* of clone TV008G65 of isolate C-98TV008 (SEQ ID NO:91).

Figure 89 presents the sequence of *Gag* of clone TV008G66 of isolate C-98TV008 (SEQ ID NO:92).

30 Figure 90 presents the sequence of *Gag* of clone TV010G74 of isolate C-98TV010 (SEQ ID NO:93).

Figure 91 presents the sequence of *Gag* of clone TV012G34 of isolate C-98TV012 (SEQ ID NO:94).

Figure 92 presents the sequence of *Gag* of clone TV012G40 of isolate C-98TV012 (SEQ ID NO:95).

5 Figure 93 presents the sequence of *Gag* of clone TV013G2 of isolate C-98TV013 (SEQ ID NO:96).

Figure 94 presents the sequence of *Gag* of clone TV013G15 of isolate C-98TV013 (SEQ ID NO:97).

10 Figure 95 presents the sequence of *Gag* of clone TV014G73 of isolate C-98TV014 (SEQ ID NO:98).

Figure 96 presents the sequence of *Gag* of clone TV018G60 of isolate C-98TV018 (SEQ ID NO:99).

Figure 97 presents the sequence of *Gag* of clone TV019G20 of isolate C-98TV019 (SEQ ID NO:100).

15 Figure 98 presents the sequence of *Gag* of clone TV019G25 of isolate C-98TV019 (SEQ ID NO:101).

Figures 99a1, 99a2, 99b and 99c depict alignments of the deduced amino acid sequences of Nef (Fig. 99a1 and 99a2), Tat (Fig. 99b) and Rev (Fig. 99c) from South African subtype C isolates (TV001 (SEQ ID NO:102 for Nef, SEQ ID NO:206, for
20 Tat and SEQ ID NO:230 for Rev); TV002 (SEQ ID NO:103, SEQ ID NO:207 for Tat and SEQ ID NO:231 for Rev); TV003 (SEQ ID NO:104 for Nef, SEQ ID NO:208 for Tat, SEQ ID NO:232 for Rev); TV004 (SEQ ID NO:105 for Nef, SEQ ID NO:209 for Tat and SEQ ID NO:233 for Rev); TV005 (SEQ ID NO:106 for Nef, SEQ ID NO:210 for Tat and SEQ ID NO:234 for Rev; TV006 (SEQ ID NO:107 for Nef, SEQ
25 ID NO:211 for Tat and SEQ ID NO:235 for Rev); TV007 (SEQ ID NO:108 for Nef, SEQ ID NO:212 for Tat and SEQ ID NO:236 for Rev); TV008 (SEQ ID NO:109 for Nef, SEQ ID NO:213 for Tat and SEQ ID NO:237 for Rev); TV010 (SEQ ID NO:110 for Nef, SEQ ID NO:214 for Tat and SEQ ID NO:238 for Rev); TV012 (SEQ ID NO:111 for Nef, SEQ ID NO:215 for Tat and SEQ ID NO:239 for Rev);
30 TV013 (SEQ ID NO:112 for Nef, SEQ ID NO:216 for Tat and SEQ ID NO:240 for Rev); TV014 (SEQ ID NO:113 for Nef, SEQ ID NO:217 for Tat and SEQ ID

NO:241 for Rev); TV018 (SEQ ID NO:114 for Nef, SEQ ID NO:218 for Tat and
 SEQ ID NO:242 for Rev); TV019 (SEQ ID NO:115 for Nef, SEQ ID NO:219 for Tat
 and SEQ ID NO:243 for Rev)) in conjunction with some subtype C reference strains
 (92BR025 (SEQ ID NO:116 for Nef, SEQ ID NO:220 for Tat and SEQ ID NO:244
 5 for Rev); 301904-Ind (SEQ ID NO:117 for Nef, SEQ ID NO:221 for Tat and SEQ ID
 NO:245 for Rev); 301905-Ind (SEQ ID NO:118 for Nef, SEQ ID NO:222 for Tat and
 SEQ ID NO:246 for Rev); 30199-Ind (SEQ ID NO:119 for Nef, SEQ ID NO:223 for
 Tat and SEQ ID NO:247 for Rev); 96BW16-D14 (SEQ ID NO:120 for Nef, SEQ ID
 NO:224 for Tat and SEQ ID NO:248 for Rev); 96BW04-09 (SEQ ID NO:121 for
 10 Nef, SEQ ID NO:225 for Tat and SEQ ID NO:249 for Rev); 96BW12-10 (SEQ ID
 NO:122 for Nef; SEQ ID NO:226 for Tat and SEQ ID NO:250 for Rev); C2220-Eth
 (SEQ ID NO:123 for Nef, SEQ ID NO:227 for Tat and SEQ ID NO:251 for Rev)) as
 well as the subtype B reference strain HXB2 (SEQ ID NO:124 for Nef, SEQ ID
 NO:228 for Tat and SEQ ID NO:252 for Rev). Consensus sequence is shown at the
 15 bottom (SEQ ID NO:125 for Nef, SEQ ID NO:229 for Tat and SEQ ID NO:253 for
 Rev). Dots represent identical residue sequences, dashes represent gaps and asterisks
 represent stop codons. Significant protein domains and conserved motifs are shaded
 and labeled.

Figure 100, sheets 1 to 9, depicts alignment of the complete Env protein from
 20 South African HIV-1 subtype C sequences (TV001c8.2 (SEQ ID NO:126);
 TV001c8.1 (SEQ ID NO:127); TV002c12.1 (SEQ ID NO:128); TV012c2.1 (SEQ ID
 NO:129); TV012c2.2 (SEQ ID NO:130); TV006c9.1 (SEQ ID NO:131); TV006cE9
 (SEQ ID NO:132); TV006c9.2 (SEQ ID NO:133); TV007cB104 (SEQ ID NO:134);
 TV007cB105 (SEQ ID NO:135); TV010cD7 (SEQ ID NO:136); TV018cF1027 (SEQ
 25 ID NO:137); TV014c6.3 (SEQ ID NO:138); TV014c6.4 (SEQ ID NO:139);
 TV008c4.3 (SEQ ID NO:140); TV008c4.4 (SEQ ID NO:141); TV019c5 (SEQ ID
 NO:142); TV003cE260 (SEQ ID NO:143); TV004cC300 (SEQ ID NO:144);
 TV013cH17 (SEQ ID NO:145); TV013cB20 (SEQ ID NO:146)) compared to the
 subtype C reference strains: IN21068 (SEQ ID NO:147), 96BW05.02 (SEQ ID
 30 NO:148), ETH2220 (SEQ ID NO:149), and 92BR025.8 (SEQ ID NO:150) from the
 Los Alamos Database. Dots denote sequence identity with the IN21068 sequence,

while dashes represent gaps introduced to optimize alignments. Carets indicate possible glycosylation sites present in most of the sequences. Asterisks show positions of cysteine residues. The V1, V2, V3, V4 and V5 variable loops, as well as the signal peptide and CD4 binding residues and sites are indicated above the sequences.

- 5 Triangles at positions 11, 25 and 35 of the V3 loop indicate amino acids assessed for SI / NSI phenotype.

Figure 101, sheets 1 to 3, depicts alignments of the deduced (A) Vif, (B), Vpr , and (C) Vpu amino acid sequences from South African subtype C isolates (in boldface, TV007-6 (SEQ ID NO:151 for Vif, SEQ ID NO:254 for Vpr and SEQ ID NO:288 for Vpu); TV007-2 (SEQ ID NO:152 for Vif, SEQ ID NO:255 for Vpr and SEQ ID NO:289 for Vpu); TV019-82 (SEQ ID NO:153 for Vif, SEQ ID NO:256 for Vpr and SEQ ID NO:290 for Vpu); TV019-85 (SEQ ID NO:154 for Vif, SEQ ID NO:257 for Vpr and SEQ ID NO:291 for Vpu); TV008-17 (SEQ NO:155 for Vif, SEQ ID NO:258 for Vpr and SEQ ID NO:292 for Vpu); TV008-1 (SEQ ID NO:156 for Vif, SEQ ID NO:259 for Vpr and SEQ ID NO:293 for Vpu); TV014-25 (SEQ ID NO:157 for Vif, SEQ ID NO:260 for Vpr and SEQ ID NO:294 for Vpu); TV014-31 (SEQ ID NO:158 for Vif, SEQ ID NO:261 for Vpr and SEQ ID NO:295 for Vpu); TV004-45 (SEQ ID NO:159 for Vif, SEQ ID NO:262 for Vpr and SEQ ID NO:296 for Vpu); TV001-2 (SEQ ID NO:160 for Vif, SEQ ID NO:263 for Vpr and SEQ ID NO:297 for Vpu); TV018-7 (SEQ ID NO:286 for Vif, SEQ ID NO:264 for Vpr and SEQ ID NO:298 for Vpu); TV018-8 (SEQ ID NO:161 for Vif, SEQ ID NO:265 for Vpr and SEQ ID NO:299 for Vpu); TV002-84 (SEQ ID NO:162 for Vif, SEQ ID NO:266 for Vpr and SEQ ID NO:300 for Vpu); TV009-3 (SEQ ID NO:163 for Vif, SEQ ID NO:267 for Vpr and SEQ ID NO:301 for Vpu); TV013-2 (SEQ ID NO:164 for Vif, SEQ ID NO:268 for Vpr and SEQ ID NO:302 for Vpu); TV013-3 (SEQ ID NO:165 for Vif, SEQ ID NO:269 for Vpr and SEQ ID NO:303 for Vpu); TV003-12 (SEQ ID NO:166 for Vif, SEQ ID NO:270 for Vpr and SEQ ID NO:304 for Vpu); TV003-B (SEQ ID NO:167 for Vif, SEQ ID NO:271 for Vpr and SEQ ID NO:305 for Vpu); TV005-81 (SEQ ID NO:168 for Vif, SEQ ID NO:272 for Vpr and SEQ ID NO:306 for Vpu); TV012-4 (SEQ ID NO:169 for Vif, SEQ ID NO:273 for Vpr and SEQ ID NO:307 for Vpu); TV006-9 (SEQ ID NO:170 for Vif, SEQ ID NO:274 for Vpr and

SEQ ID NO:308 for Vpu); TV010-25 (SEQ ID NO:171 for Vif, SEQ ID NO:275 for Vpr and SEQ ID NO:309 for Vpu) in conjunction with some subtype C reference strains 92BR025 (SEQ ID NO:172 for Vif, SEQ ID NO:276 for Vpr and SEQ ID NO:310 for Vpu); 301904-Ind (SEQ ID NO:173 for Vif, SEQ ID NO:277 for Vpr and SEQ ID NO:311 for Vpu); 301905-Ind (SEQ ID NO:174 for Vif, SEQ ID NO:278 for Vpr and SEQ ID NO:312 for Vpu); 30199-Ind (SEQ ID NO:175 for Vif, SEQ ID NO:279 for Vpr and SEQ ID NO:313 for Vpu); 96BW16-D14 (SEQ ID NO:176 for Vif, SEQ ID NO:280 for Vpr and SEQ ID NO:314 for Vpu); 96BW04-09 (SEQ ID NO:177 for Vif, SEQ ID NO:281 for Vpr and SEQ ID NO:315 for Vpu); 96BW12-10 (SEQ ID NO:178 for Vif, SEQ ID NO:282 for Vpr and SEQ ID NO:316 for Vpu); C2220-Eth (SEQ ID NO:179 for Vif, SEQ ID NO:283 for Vpr and SEQ ID NO:317 for Vpu)) as well as HXB2 (SEQ ID NO:180 for Vif, SEQ ID NO:284 for Vpr and SEQ ID NO:318 for Vpu). Consensus sequences are shown as SEQ ID NO:287 for Vif, SEQ ID NO:285 for Vpr and SEQ ID NO:319 for Vpu.

Figure 102, sheets 1 and 2, depicts the nucleotide sequence of from the 3' region of the clone designated 8_2_TV1 (SEQ ID NO:181).

Figure 103, sheets 1 to 5, depicts the nucleotide sequence of 2_1/4_TV12_C_ZA (SEQ ID NO:182).

Figure 104 depicts the nucleotide sequence of gp140.modTV1.mut1.dV2 (SEQ ID NO:183).

Figure 105 depicts the nucleotide sequence of gp140mod.TV1.mut2.dV2 (SEQ ID NO:184).

Figure 106 depicts the nucleotide sequence of gp140mod.TV1.mut3.dV2 (SEQ ID NO:185).

Figure 107 depicts the nucleotide sequence of gp140mod.TV1.mut4.dV2 (SEQ ID NO:186).

Figure 108 depicts the nucleotide sequence of gp140.mod.TV1.GM161 (SEQ ID NO:187).

Figure 109 depicts the nucleotide sequence of gp140mod.TV1.GM161-195-204 (SEQ ID NO:188).

Figure 110 depicts the nucleotide sequence of gp140mod.TV1.GM161-204 (SEQ ID NO:189).

Figure 111 depicts the nucleotide sequence of gp140mod.TV1.GM-V1V2 (SEQ ID NO:190).

5 Figure 112 depicts the nucleotide sequence of
gp140modC8.2mut7.delV2.Kozmod.Ta (SEQ ID NO:191).

Figure 113 depicts alignment of the amino acid sequences of various Env cleavage site mutants (translation of gp140mod.TV1.delV2 (SEQ ID NO:192); translation of gp140mod.TV1.mut1.dV2 (SEQ ID NO:193); translation of
10 gp140mod.TV1.mut2.dV2 (SEQ ID NO:194); translation of
gp140mod.TV1.mut3.dV2 (SEQ ID NO:195); translation of
gp140mod.TV1.mut4.dV2 (SEQ ID NO:196); and translation of
gp140mod.TV1.mut7.dV2 (SEQ ID NO:197)). Amino acid changes are shown in
bold.

15 Figure 114 depicts alignment of amino acid sequences of various Env glycosylation mutants (GM), including translation of gp140mod.TV1 (SEQ ID NO:198); translation of gp140mod.TV1.GM161 (SEQ ID NO:199); translation of gp140mod.TV1.GM161-204 (SEQ ID NO:200); translation of gp140mod.TV1.GM161-195-204 (SEQ ID NO:201); and translation of
20 gp140mod.TV1.GM-V1V2 (SEQ ID NO:202).

Figure 115 depicts the nucleotide sequence of Nef-myrD124LLAA (SEQ ID NO:203).

Figure 116 depicts the amino acid sequence of the protein translated (SEQ ID NO:204) from Nef-myrD124LLAA.

25 Figure 117 depicts the nucleotide sequence of gp160mod.TV2 (SEQ ID NO:205).

Figure 118 presents an overview of genome organization of HIV-1 and useful subgenomic fragments.

Figure 119 is a graph depicting log geometric mean antibody titers in
30 immunized rabbits following immunization with Env DNA and protein.

Figure 120 is a bar graph depicting comparison of ELISA titers against subtype B and C Env proteins in rabbit sera collected after 3 DNA immunizations and a single protein boost.

Figure 121 presents data of neutralizing antibody responses against subtype B
5 SF162 EnvD V2 strain in rabbits immunized with subtype C TV1 Env in a DNA prime protein boost regimen.

Figure 122 presents data of neutralizing antibody responses against subtype C primary strains, TV1 and TV2 in 5.25 reporter cell assay after a single protein boost.

Figure 123 presents data of neutralizing antibody responses against subtype C,
10 TV1 and Du174, and subtype B, SF162 after a single protein boost (as measured by Duke PBMC assay).

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated,
15 conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental*
20 *Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to*
25 *Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag).

As used in this specification, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents.

1. DEFINITIONS

30 In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

“Synthetic” sequences, as used herein, refers to HIV polypeptide-*encoding* polynucleotides whose expression has been modified as described herein, for example, by codon substitution, altered activities, and/or inactivation of inhibitory sequences.

“Wild-type” or “native” sequences, as used herein, refers to polypeptide encoding

5 sequences that are essentially as they are found in nature, e.g., Gag, Pol, Vif, Vpr, Tat, Rev, Vpu, Env and/or Nef encoding sequences as found in HIV isolates, e.g., SF162, SF2, AF110965, AF110967, AF110968, AF110975, 8_5_TV1_C.ZA, 8_2_TV1_C.ZA or 12-5_1_TV2_C.ZA. The various regions of the HIV genome are shown in Table A, with numbering relative to 8_5_TV1_C.ZA (Figures 1A-1D).

10 Thus, the term “Pol” refers to one or more of the following polypeptides: polymerase (p6Pol); protease (prot); reverse transcriptase (p66RT or RT); RNaseH (p15RNaseH); and/or integrase (p31Int or Int). Identification of gene regions for any selected HIV isolate can be performed by one of ordinary skill in the art based on the teachings presented herein and the information known in the art, for example, by

15 performing alignments relative to 8_5_TV1_C.ZA (Figures 1A-1D) or alignment to other known HIV isolates, for example, Subtype B isolates with gene regions (e.g., SF2, GenBank Accession number K02007; SF162, GenBank Accession Number M38428) and Subtype C isolates with gene regions (e.g., GenBank Accession Number AF110965 and GenBank Accession Number AF110975).

20 As used herein, the term “virus-like particle” or “VLP” refers to a nonreplicating, viral shell, derived from any of several viruses discussed further below. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form

25 spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art and discussed more fully below. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, X-ray crystallography, and the like. See, e.g., Baker et al.,

30 *Biophys. J.* (1991) 60:1445-1456; Hagensee et al., *J. Virol.* (1994) 68:4503-4505. For example, VLPs can be isolated by density gradient centrifugation and/or identified

by characteristic density banding. Alternatively, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

By "particle-forming polypeptide" derived from a particular viral protein is meant a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletions, which has the ability to form VLPs under conditions that favor VLP formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also includes deletions, additions and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP. Preferred substitutions are those which are conservative in nature, i.e., those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids.

The term "HIV polypeptide" refers to any amino acid sequence that exhibits sequence homology to native HIV polypeptides (*e.g.*, Gag, Env, Prot, Pol, RT, Int, vif, vpr, vpu, tat, rev, nef and/or combinations thereof) and/or which is functional. Non-limiting examples of functions that may be exhibited by HIV polypeptides include, use as immunogens (*e.g.*, to generate a humoral and/or cellular immune response), use in diagnostics (*e.g.*, bound by suitable antibodies for use in ELISAs or other immunoassays) and/or polypeptides which exhibit one or more biological activities associated with the wild type or synthetic HIV polypeptide. For example, as used herein, the term "Gag polypeptide" may refer to a polypeptide that is bound by one or

more anti-Gag antibodies; elicits a humoral and/or cellular immune response; and/or exhibits the ability to form particles.

An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen." Normally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes. Antibodies such as anti-idiotypic antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

For purposes of the present invention, antigens can be derived from any of several known viruses, bacteria, parasites and fungi, as described more fully below. The term also intends any of the various tumor antigens. Furthermore, for purposes of the present invention, an "antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to an immune response mediated by antibody molecules, while a

“cellular immune response” is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells (“CTL”s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A “cellular immune response” also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376. Recent methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells (e.g., by the tetramer technique)(reviewed by McMichael, A.J., and O’Callaghan, C.A., *J. Exp. Med.* **187**(9)1367-1371, 1998; Mcheyzer-Williams, M.G., et al, *Immunol. Rev.* **150**:5-21, 1996; Lalvani, A., et al, *J. Exp. Med.* **186**:859-865, 1997).

Thus, an immunological response as used herein may be one which stimulates the production of CTLs, and/or the production or activation of helper T- cells. The

antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor T-cells and/or $\gamma\delta$ T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest. The immunogenic composition can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal (*e.g.*, intra-rectally or intra-vaginally) administration.

By "subunit vaccine" is meant a vaccine composition which includes one or more selected antigens but not all antigens, derived from or homologous to, an antigen from a pathogen of interest such as from a virus, bacterium, parasite or fungus. Such a composition is substantially free of intact pathogen cells or pathogenic particles, or the lysate of such cells or particles. Thus, a "subunit vaccine" can be prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or analogs thereof. The method of obtaining an antigen included in the subunit vaccine can thus include standard purification techniques, recombinant production, or synthetic production.

"Substantially purified" general refers to isolation of a substance (compound, polynucleotide, protein, polypeptide, polypeptide composition) such that the substance comprises the majority percent of the sample in which it resides. Typically in a sample a substantially purified component comprises 50%, preferably 80%-85%, more preferably 90-95% of the sample. Techniques for purifying polynucleotides and polypeptides of interest are well-known in the art and include, for example, ion-exchange chromatography, affinity chromatography and sedimentation according to density.

A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral or procaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence such as a stop codon may be located 3' to the coding sequence.

Typical "control elements", include, but are not limited to, transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), and translation termination sequences. For example, the sequences and/or vectors described herein may also include one or more additional sequences that may optimize translation and/or termination including, but not limited to, a Kozak sequence (*e.g.*, GCCACC, nucleotides 1 to 6 of SEQ ID NO:191) placed in front (5') of the ATG of the codon-optimized wild-type leader or any other suitable leader sequence (*e.g.*, tpa1, tpa2, wtLnat (native wild-type leader)) or a termination sequence (*e.g.*, TAA or, preferably, TAAA, nucleotides 1978 to 1981 of SEQ ID NO:191) placed after (3') the coding sequence.

A "polynucleotide coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon, for example, at or near the 5' terminus and a translation stop codon, for example, at or near the 3' terminus. Exemplary coding sequences are the modified viral polypeptide-coding sequences of the present invention. The coding regions of the polynucleotide sequences of the present invention are identifiable by one of skill in the art and may, for example, be

easily identified by performing translations of all three frames of the polynucleotide and identifying the frame corresponding to the encoded polypeptide, for example, a synthetic nef polynucleotide of the present invention encodes a nef-derived polypeptide. A transcription termination sequence may be located 3' to the coding sequence. Typical "control elements", include, but are not limited to, transcription regulators, such as promoters, transcription enhancer elements, transcription termination signals, and polyadenylation sequences; and translation regulators, such as sequences for optimization of initiation of translation, *e.g.*, Shine-Dalgarno (ribosome binding site) sequences, Kozak sequences (*i.e.*, sequences for the optimization of translation, located, for example, 5' to the coding sequence), leader sequences, translation initiation codon (*e.g.*, ATG), and translation termination sequences. In certain embodiments, one or more translation regulation or initiation sequences (*e.g.*, the leader sequence) are derived from wild-type translation initiation sequences, *i.e.*, sequences that regulate translation of the coding region in their native state. Wild-type leader sequences that have been modified, using the methods described herein, also find use in the present invention. Promoters can include inducible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), repressible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and constitutive promoters.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet

transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a
5 polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by
10 expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original
15 cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence
20 encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical
25 or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and
30 determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide

or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

Two or more polynucleotide sequences can be compared by determining their “percent identity.” Two or more amino acid sequences likewise can be compared by
5 determining their “percent identity.” The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in*
10 *Applied Mathematics* 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic
15 acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their BestFit utility application. The default parameters for this method are described in the *Wisconsin Sequence Analysis Package Program Manual, Version 8* (1995) (available from Genetics Computer Group, Madison, WI). Other equally suitable programs for calculating the percent identity or similarity between sequences
20 are generally known in the art.

For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions. Another method of establishing percent identity in the context of the present invention is to use
25 the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six).
30 From the data generated, the “Match” value reflects “sequence identity.” Other suitable programs for calculating the percent identity or similarity between sequences

are generally known in the art, such as the alignment program BLAST, which can also be used with default parameters. For example, BLASTN and BLASTP can be used with the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; 5 sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

One of skill in the art can readily determine the proper search parameters to use 10 for a given sequence, exemplary preferred Smith Waterman based parameters are presented above. For example, the search parameters may vary based on the size of the sequence in question. Thus, for the polynucleotide sequences of the present invention the length of the polynucleotide sequence disclosed herein is searched against a selected database and compared to sequences of essentially the same length to 15 determine percent identity. For example, a representative embodiment of the present invention would include an isolated polynucleotide comprising X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least about a selected level of percent identity relative to Y contiguous nucleotides of one or more of the sequences described herein (e.g., in Table C) or fragment thereof, and (ii) for search 20 purposes X equals Y, wherein Y is a selected reference polynucleotide of defined length (for example, a length of from 15 nucleotides up to the number of nucleotides present in a selected full-length sequence).

The sequences of the present invention can include fragments of the sequences, for example, from about 15 nucleotides up to the number of nucleotides present in the 25 full-length sequences described herein (e.g., see the Figures), including all integer values falling within the above-described range. For example, fragments of the polynucleotide sequences of the present invention may be 30-60 nucleotides, 60-120 nucleotides, 120-240 nucleotides, 240-480 nucleotides, 480-1000 nucleotides, and all integer values therebetween.

30 The synthetic expression cassettes (and purified polynucleotides) of the present invention include related polynucleotide sequences having about 80% to 100%, greater

than 80-85%, preferably greater than 90-92%, more preferably greater than 95%, and most preferably greater than 98% up to 100% (including all integer values falling within these described ranges) sequence identity to the synthetic expression cassette and/or polynucleotide sequences disclosed herein (for example, to the sequences of the present invention) when the sequences of the present invention are used as the query sequence against, for example, a database of sequences.

Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution hybridization, or the like, see Sambrook, et al., *supra* or Ausubel et al., *supra*). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that lacks even a partial degree of sequence identity (for example, a probe having less than about 30% sequence identity with the target molecule), such that, in the absence of non-specific binding events, the secondary probe will not hybridize to the target.

When utilizing a hybridization-based detection system, a nucleic acid probe is chosen that is complementary to a target nucleic acid sequence, and then by selection of appropriate conditions the probe and the target sequence "selectively hybridize," or bind, to each other to form a hybrid molecule. A nucleic acid molecule that is capable of hybridizing selectively to a target sequence under "moderately stringent" typically hybridizes under conditions that allow detection of a target nucleic acid sequence of at least about 10-14 nucleotides in length having at least approximately 70% sequence identity with the sequence of the selected nucleic acid probe. Stringent hybridization conditions typically allow detection of target nucleic acid sequences of at least about 10-14 nucleotides in length having a sequence identity of greater than about 90-95% with the sequence of the selected nucleic acid probe. Hybridization conditions useful

for probe/target hybridization where the probe and target have a specific degree of sequence identity, can be determined as is known in the art (see, for example, Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington, DC; IRL Press).

5 With respect to stringency conditions for hybridization, it is well known in the art that numerous equivalent conditions can be employed to establish a particular stringency by varying, for example, the following factors: the length and nature of probe and target sequences, base composition of the various sequences, concentrations of salts and other hybridization solution components, the presence or absence of
10 blocking agents in the hybridization solutions (e.g., formamide, dextran sulfate, and polyethylene glycol), hybridization reaction temperature and time parameters, as well as, varying wash conditions. The selection of a particular set of hybridization conditions is selected following standard methods in the art (see, for example, Sambrook; et al., *supra* or Ausubel et al., *supra*).

15 A first polynucleotide is "derived from" second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above.

 A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by
20 a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above.

 Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to
25 polypeptides of that virus as described above.

 "Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide
30 encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence.

Further, polyproteins can be constructed by fusing in-frame two or more polynucleotide sequences encoding polypeptide or peptide products. Further, polycistronic coding sequences may be produced by placing two or more polynucleotide sequences encoding polypeptide products adjacent each other, typically
5 under the control of one promoter, wherein each polypeptide coding sequence may be modified to include sequences for internal ribosome binding sites.

“Purified polynucleotide” refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which
10 the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

15 By “nucleic acid immunization” is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into
20 cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

“Gene transfer” or “gene delivery” refers to methods or systems for reliably inserting DNA of interest into a host cell. Such methods can result in transient
25 expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors
30 may be referred to as vaccines or vaccine vectors.

“T lymphocytes” or “T cells” are non-antibody producing lymphocytes that constitute a part of the cell-mediated arm of the immune system. T cells arise from immature lymphocytes that migrate from the bone marrow to the thymus, where they undergo a maturation process under the direction of thymic hormones. Here, the
5 mature lymphocytes rapidly divide increasing to very large numbers. The maturing T cells become immunocompetent based on their ability to recognize and bind a specific antigen. Activation of immunocompetent T cells is triggered when an antigen binds to the lymphocyte's surface receptors.

The term “transfection” is used to refer to the uptake of foreign DNA by a cell.
10 A cell has been “transfected” when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al. (1989) *Molecular Cloning, a laboratory manual*, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene*
15 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells. The term refers to both stable and transient uptake of the genetic material, and includes uptake of peptide- or antibody-linked DNAs.

A “vector” is capable of transferring gene sequences to target cells (e.g., viral vectors, non-viral vectors, particulate carriers, and liposomes). Typically, “vector
20 construct,” “expression vector,” and “gene transfer vector,” mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Transfer of a “suicide gene” (e.g., a drug-susceptibility gene) to a target cell
25 renders the cell sensitive to compounds or compositions that are relatively nontoxic to normal cells. Moolten, F.L. (1994) *Cancer Gene Ther.* 1:279-287. Examples of suicide genes are thymidine kinase of herpes simplex virus (HSV-tk), cytochrome P450 (Manome et al. (1996) *Gene Therapy* 3:513-520), human deoxycytidine kinase (Manome et al. (1996) *Nature Medicine* 2(5):567-573) and the bacterial enzyme
30 cytosine deaminase (Dong et al. (1996) *Human Gene Therapy* 7:713-720). Cells which express these genes are rendered sensitive to the effects of the relatively

nontoxic prodrugs ganciclovir (HSV-tk), cyclophosphamide (cytochrome P450 2B1), cytosine arabinoside (human deoxycytidine kinase) or 5-fluorocytosine (bacterial cytosine deaminase). Culver et al. (1992) *Science* 256:1550-1552, Huber et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:8302-8306.

5 A “selectable marker” or “reporter marker” refers to a nucleotide sequence included in a gene transfer vector that has no therapeutic activity, but rather is included to allow for simpler preparation, manufacturing, characterization or testing of the gene transfer vector.

10 A “specific binding agent” refers to a member of a specific binding pair of molecules wherein one of the molecules specifically binds to the second molecule through chemical and/or physical means. One example of a specific binding agent is an antibody directed against a selected antigen.

15 By “subject” is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as rhesus macaque, chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular
20 age. Thus, both adult and newborn individuals are intended to be covered. The system described above is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

25 By “pharmaceutically acceptable” or “pharmacologically acceptable” is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

30 By “physiological pH” or a “pH in the physiological range” is meant a pH in the range of approximately 7.0 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, "treatment" refers to any of (i) the prevention of infection or reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, and (iii) the substantial or complete elimination of the pathogen in question. Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

By "co-administration" is meant administration of more than one composition or molecule. Thus, co-administration includes concurrent administration or sequentially administration (in any order), via the same or different routes of administration. Non-limiting examples of co-administration regimes include, co-administration of nucleic acid and polypeptide; co-administration of different nucleic acids (*e.g.*, different expression cassettes as described herein and/or different gene delivery vectors); and co-administration of different polypeptides (*e.g.*, different HIV polypeptides and/or different adjuvants). The term also encompasses multiple administrations of one of the co-administered molecules or compositions (*e.g.*, multiple administrations of one or more of the expression cassettes described herein followed by one or more administrations of a polypeptide-containing composition). In cases where the molecules or compositions are delivered sequentially, the time between each administration can be readily determined by one of skill in the art in view of the teachings herein.

"Lentiviral vector", and "recombinant lentiviral vector" refer to a nucleic acid construct which carries, and within certain embodiments, is capable of directing the expression of a nucleic acid molecule of interest. The lentiviral vector include at least one transcriptional promoter/enhancer or locus defining element(s), or other elements which control gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional modification of protein. Such vector constructs must also include a packaging signal, long terminal repeats (LTRS) or portion thereof, and positive and negative strand primer binding sites appropriate to the retrovirus used (if these are not already present in the retroviral vector). Optionally, the recombinant lentiviral vector may also include a signal which directs polyadenylation, selectable markers such as Neo, TK, hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites

and a translation termination sequence. By way of example, such vectors typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3'LTR or a portion thereof

5 “Lentiviral vector particle” as utilized within the present invention refers to a lentivirus which carries at least one gene of interest. The retrovirus may also contain a selectable marker. The recombinant lentivirus is capable of reverse transcribing its genetic material (RNA) into DNA and incorporating this genetic material into a host cell's DNA upon infection. Lentiviral vector particles may have a lentiviral envelope, a non-lentiviral envelope (e.g., an amphi or VSV-G envelope), or a chimeric envelope.

10 “Nucleic acid expression vector” or “Expression cassette” refers to an assembly which is capable of directing the expression of a sequence or gene of interest. The nucleic acid expression vector includes a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. Expression cassettes described herein may be contained within a plasmid construct. In
15 addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a “mammalian” origin of replication (e.g., a SV40 or adenovirus origin of replication).

20 “Packaging cell” refers to a cell which contains those elements necessary for production of infectious recombinant retrovirus which are lacking in a recombinant retroviral vector. Typically, such packaging cells contain one or more expression cassettes which are capable of expressing proteins which encode *Gag*, *pol* and *env* proteins.

25 “Producer cell” or “vector producing cell” refers to a cell which contains all elements necessary for production of recombinant retroviral vector particles.

2. MODES OF CARRYING OUT THE INVENTION

30 Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the

purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

2.1.0. THE HIV GENOME

The HIV genome and various polypeptide-encoding regions are shown in Table A. The nucleotide positions are given relative to 8_5_TV1_C.ZA (Figure 1; an HIV Type C isolate). However, it will be readily apparent to one of ordinary skill in the art in view of the teachings of the present disclosure how to determine corresponding regions in other HIV strains or variants (*e.g.*, isolates HIV_{IIIb}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (*e.g.*, subtypes, A through G, and O), HIV-2 strains and diverse subtypes (*e.g.*, HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, *e.g.*, Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991); *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA; for a description of these and other related viruses), using for example, sequence comparison programs (*e.g.*, BLAST and others described herein) or identification and alignment of structural features (*e.g.*, a program such as the "ALB" program described herein that can identify the various regions).

Table A: Regions of the HIV Genome relative to 8_5_TV1_C.ZA

25	Region	Position in nucleotide sequence
	5'LTR	1-636
	U3	1-457
	R	458-553
	U5	554-636
30	NFkB II	340-348
	NFkB I	354-362
	Sp1 III	379-388
	Sp1 II	390-398

	Sp1 I	400-410
	TATA Box	429-433
	TAR	474-499
	Poly A signal	529-534
5	PBS	638-655
	p7 binding region, packaging signal	685-791
10	Gag:	792-2285
	p17	792-1178
	p24	1179-1871
	Cyclophilin A bdg.	1395-1505
	MHR	1632-1694
15	p2	1872-1907
	p7	1908-2072
	Frameshift slip	2072-2078
	p1	2073-2120
	p6Gag	2121-2285
20	Zn-motif I	1950-1991
	Zn-motif II	2013-2054
	Pol:	2072-5086
	p6Pol	2072-2245
25	Prot	2246-2542
	p66RT	2543-4210
	p15RNaseH	3857-4210
	p31Int	4211-5086
30	Vif:	5034-5612
	Hydrophilic region	5292-5315
	Vpr:	5552-5839
	Oligomerization	5552-5677
35	Amphipathic a-helix	5597-5653
	Tat:	5823-6038 and 8417-8509
	Tat-1 exon	5823-6038
	Tat-2 exon	8417-8509
40	N-terminal domain	5823-5885

	Trans-activation domain	5886-5933
	Transduction domain	5961-5993
	Rev:	5962-6037 and 8416-8663
5	Rev-1 exon	5962-6037
	Rev-2 exon	8416-8663
	High-affinity bdg. site	8439-8486
	Leu-rich effector domain	8562-8588
10	Vpu:	6060-6326
	Transmembrane domain	6060-6161
	Cytoplasmic domain	6162-6326
	Env (gp160):	6244-8853
15	Signal peptide	6244-6324
	gp120	6325-7794
	V1	6628-6729
	V2	6727-6852
	V3	7150-7254
20	V4	7411-7506
	V5	7663-7674
	C1	6325-6627
	C2	6853-7149
	C3	7255-7410
25	C4	7507-7662
	C5	7675-7794
	CD4 binding	7540-7566
	gp41	7795-8853
	Fusion peptide	7789-7842
30	Oligomerization domain	7924-7959
	N-terminal heptad repeat	7921-8028
	C-terminal heptad repeat	8173-8280
	Immunodominant region	8023-8076
35	Nef:	8855-9478
	Myristoylation	8858-8875
	SH3 binding	9062-9091
	Polypurine tract	9128-9154
40	SH3 binding	9296-9307

It will be readily apparent that one of skill in the art can readily align any sequence to that shown in Table A to determine relative locations of any particular HIV gene. For example, using one of the alignment programs described herein (*e.g.*, BLAST), other HIV genomic sequences can be aligned with 8_5_TV1_C.ZA (Table A) and locations of genes determined. Polypeptide sequences can be similarly aligned. For example, Figures 2A-2C shows the alignment of Env polypeptide sequences from various strains, relative to SF-162. As described in detail in co-owned WO/39303, Env polypeptides (*e.g.*, gp120, gp140 and gp160) include a "bridging sheet" comprised of 4 anti-parallel β -strands (β -2, β -3, β -20 and β -21) that form a β -sheet. Extruding from one pair of the β -strands (β -2 and β -3) are two loops, V1 and V2. The β -2 sheet occurs at approximately amino acid residue 113 (Cys) to amino acid residue 117 (Thr) while β -3 occurs at approximately amino acid residue 192 (Ser) to amino acid residue 194 (Ile), relative to SF-162. The "V1/V2 region" occurs at approximately amino acid positions 120 (Cys) to residue 189 (Cys), relative to SF-162. Extruding from the second pair of β -strands (β -20 and β -21) is a "small-loop" structure, also referred to herein as "the bridging sheet small loop." The locations of both the small loop and bridging sheet small loop can be determined relative to HXB-2 following the teachings herein and in WO/39303. Also shown by arrows in Figure 2A-C are approximate sites for deletions sequence from the beta sheet region. The "*" denotes N-glycosylation sites that can be mutated following the teachings of the present specification.

2.1.1. WILD-TYPE HIV SEQUENCES

Isolated nucleotide sequences for various novel subtype C novel isolates are shown in Table A1 below. Sequence were obtained and analyzed (*e.g.*, phylogenetic tree analysis) as described in Engelbrecht et al (2001) *AIDS Res. Hum. Retroviruses* 17(16):1533-1547. (See, also, GenBank). Sequences of accessory proteins and analysis of these sequences is described in Scriba et al. (2001) *AIDS Res. Hum. Retroviruses* 17(8):775-781.

Table A1: Wild-Type Sequences

Name	SEQ ID NO	Figure Number	Description
<i>Env</i> TV001c8.2	61	58 (2 sheets)	complete <i>Env</i> sequence of clone TV001c8.2 of isolate C-98TV001
<i>Env</i> TV001c8.5	62	59 (2 sheets)	complete <i>Env</i> sequence of clone TV001c8.5 of isolate C-98TV001
<i>Env</i> TV001c12.1	63	60 (2 sheets)	complete <i>Env</i> sequence of clone TV001c12.1 of isolate C-98TV002
<i>Env</i> TV003cE260	64	61 (2 sheets)	complete <i>Env</i> sequence of clone TV003cE260 of isolate C-98TV003
<i>Env</i> TV004cC300	65	62 (2 sheets)	complete <i>Env</i> sequence of clone TV004cC300 of isolate C-98TV004
<i>Env</i> TV006c9.1	66	63 (2 sheets)	complete <i>Env</i> sequence of clone TV006c9.1 of isolate C-98TV006
<i>Env</i> TV006c9.2	67	64 (2 sheets)	complete <i>Env</i> sequence of clone TV006c9.2 of isolate C-98TV006
<i>Env</i> TV006cE9	68	65 (2 sheets)	complete <i>Env</i> sequence of clone TV006cE9 of isolate C-98TV006
<i>Env</i> TV007cB104	69	66 (2 sheets)	complete <i>Env</i> sequence of clone TV007cB104 of isolate C-98TV007
<i>Env</i> TV007cB105	70	67 (2 sheets)	complete <i>Env</i> sequence of clone TV007cB105 of isolate C-98TV007
<i>Env</i> TV008c4.3	71	68 (2 sheets)	complete <i>Env</i> sequence of clone TV008c4.3 of isolate C-98TV008
<i>Env</i> TV008c4.4	72	69 (2 sheets)	complete <i>Env</i> sequence of clone TV008c4.4 of isolate C-98TV008
<i>Env</i> TV010cD7	73	70 (2 sheets)	complete <i>Env</i> sequence of clone TV010cD7 of isolate C-98TV010
<i>Env</i> TV012c2.1	74	71 (2 sheets)	complete <i>Env</i> sequence of clone TV012c2.1 of isolate C-98TV012
<i>Env</i> TV012c2.2	75	72 (2 sheets)	complete <i>Env</i> sequence of clone TV012c2.2 of isolate C-98TV012
<i>Env</i> TV013cB20	76	73 (2 sheets)	complete <i>Env</i> sequence of clone TV013cB20 of isolate C-98TV013

	Name	SEQ ID NO	Figure Number	Description
	<i>Env</i> TV013cH17	77	74 (2 sheets)	complete <i>Env</i> sequence of clone TV013cH17 of isolate C-98TV013
	<i>Env</i> TV014c6.3	78	75 (2 sheets)	complete <i>Env</i> sequence of clone TV014c6.3 of isolate C-98TV014
	<i>Env</i> TV014c6.4	79	76 (2 sheets)	complete <i>Env</i> sequence of clone TV014c6.4 of isolate C-98TV014
	<i>Env</i> TV018cF1027	80	77 (2 sheets)	complete <i>Env</i> sequence of clone TV018cF1027 of isolate C-98TV018
5	<i>Env</i> TV019c5	81	78 (2 sheets)	complete <i>Env</i> sequence of clone TV019c5 of isolate C-98TV019
	<i>Gag</i> TV001G8	82	79	complete <i>Gag</i> sequence of clone TV001G8 of isolate C-98TV001
	<i>Gag</i> TV001G11	83	80	complete <i>Gag</i> sequence of clone TV001G11 of isolate C-98TV001
	<i>Gag</i> TV002G8	84	81	complete <i>Gag</i> sequence of clone TV002G8 of isolate C-98TV002
	<i>Gag</i> TV003G15	85	82	complete <i>Gag</i> sequence of clone TV003G15 of isolate C-98TV003
10	<i>Gag</i> TV004G17	86	83	complete <i>Gag</i> sequence of clone TV004G17 of isolate C-98TV004
	<i>Gag</i> TV004G24	87	84	complete <i>Gag</i> sequence of clone TV004G24 of isolate C-98TV004
	<i>Gag</i> TV006G11	88	85	complete <i>Gag</i> sequence of clone TV006G11 of isolate C-98TV006
	<i>Gag</i> TV006G97	89	86	complete <i>Gag</i> sequence of clone TV006G97 of isolate C-98TV006
	<i>Gag</i> TV007G59	90	87	complete <i>Gag</i> sequence of clone TV007G59 of isolate C-98TV009
15	<i>Gag</i> TV008G65	91	88	complete <i>Gag</i> sequence of clone TV008G65 of isolate C-98TV008
	<i>Gag</i> TV008G66	92	89	complete <i>Gag</i> sequence of clone TV008G66 of isolate C-98TV008

Name	SEQ ID NO	Figure Number	Description
<i>Gag</i> TV010G74	93	90	complete <i>Gag</i> sequence of clone TV010G74 of isolate C-98TV010
<i>Gag</i> TV012G34	94	91	complete <i>Gag</i> sequence of clone TV012G34 of isolate C-98TV012
<i>Gag</i> TV012G40	95	92	complete <i>Gag</i> sequence of clone TV012G40 of isolate C-98TV012
<i>Gag</i> TV013G2	96	93	complete <i>Gag</i> sequence of clone TV013G2 of isolate C-98TV013
<i>Gag</i> TV013G15	97	94	complete <i>Gag</i> sequence of clone TV013G15 of isolate C-98TV013
<i>Gag</i> TV014G73	98	95	complete <i>Gag</i> sequence of clone TV014G73 of isolate C-98TV014
<i>Gag</i> TV018G60	99	96	complete <i>Gag</i> sequence of clone TV018G60 of isolate C-98TV018
<i>Gag</i> TV019G20	100	97	complete <i>Gag</i> sequence of clone TV019G20 of isolate C-98TV019
<i>Gag</i> TV019G25	101	98	complete <i>Gag</i> sequence of clone TV019G25 of isolate C-98TV019
8_2_TV1 LTR	181	102 (2 sheets)	sequence from the 3' region of the clone designated 8_2_TV1
2_1/4_TV12_C_ZA	182	103 (5 sheets)	sequence of 2_1/4_TV12_C_ZA

2.2.0 SYNTHETIC EXPRESSION CASSETTES

One aspect of the present invention is the generation of HIV-1 coding sequences, and related sequences, for example having improved expression relative to the corresponding wild-type sequences.

2.2.1 MODIFICATION OF HIV-1 NUCLEIC ACID CODING SEQUENCES

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. The HIV codon usage reflects a high content of the nucleotides A or T

of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The HIV coding sequences were modified to be comparable to codon usage found in highly expressed human genes.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of, for example, the Gag coding sequences. The RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements can be inactivated by introducing multiple point mutations that do not alter the reading frame of the encoded proteins.

Third, for some genes the coding sequence has been altered such that the polynucleotide coding sequence encodes a gene product that is inactive or non-functional (e.g., inactivated polymerase, protease, tat, rev, nef, vif, vpr, and/or vpu gene products). Example 1 describes some exemplary mutations. Example 8 presents information concerning functional analysis of mutated Tat, Rev and Nef antigens.

The synthetic coding sequences are assembled by methods known in the art, for example by companies such as the Midland Certified Reagent Company (Midland, Texas).

Modification of the Gag polypeptide coding sequences results in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells).

Some exemplary polynucleotide sequences encoding Gag-containing polypeptides are GagComplPolmut_C, GagComplPolmutAtt_C, GagComplPolmutIna_C, GagComplPolmutInaTatRevNef_C, GagPolmut_C, GagPolmutAtt_C, GagPolmutIna_C, GagProtInaRTmut_C, GagProtInaRTmutTatRevNef_C, GagRTmut_C, GagRTmutTatRevNef_C, GagTatRevNef_C, and gp120mod.TV1.del118-210.

Similarly, the present invention also includes synthetic Env-encoding polynucleotides and modified Env proteins, for example, gp120mod.TV1.del118-210,

gp120mod.TV1.delV1V2, gp120mod.TV1.delV2, gp140mod.TV1.del118-210,
 gp140mod.TV1.delV1V2, gp140mod.TV1.delV2, gp140mod.TV1.mut7,
 gp140mod.TV1.tpa2, gp140TMmod.TV1, gp160mod.TV1.del118-210,
 gp160mod.TV1.delV1V2, gp160mod.TV1.delV2, gp160mod.TV1.dV1,
 5 gp160mod.TV1.dV1-gagmod.BW965, gp160mod.TV1.dV1V2-gagmod.BW965,
 gp160mod.TV1.dV2-gagmod.BW965, gp160mod.TV1.tpa2, and gp160mod.TV1-
 gagmod.BW965.

The codon usage pattern for Env was modified as described above for Gag so
 that the resulting nucleic acid coding sequence was comparable to codon usage found
 10 in highly expressed human genes. Experiments performed in support of the present
 invention show that the synthetic Env sequences were capable of higher level of
 protein production relative to the native Env sequences.

Modification of the Env polypeptide coding sequences results in improved
 expression relative to the wild-type coding sequences in a number of mammalian cell
 15 lines (as well as other types of cell lines, including, but not limited to, insect cells).
 Similar Env polypeptide coding sequences can be obtained, modified and tested for
 improved expression from a variety of isolates, including those described above for
 Gag.

Further modifications of Env include, but are not limited to, generating
 20 polynucleotides that encode Env polypeptides having mutations and/or deletions
 therein. For instance, the hypervariable regions, V1 and/or V2, can be deleted as
 described herein. Additionally, other modifications, for example to the bridging sheet
 region and/or to N-glycosylation sites within Env can also be performed following the
 teachings of the present specification. (see, Figure2A-C, as well as WO 00/39303,
 25 WO 00/39302, WO 00/39304, WO 02/04493). Various combinations of these
 modifications can be employed to generate synthetic expression cassettes as described
 herein.

The present invention also includes expression cassettes which include
 synthetic Pol sequences. As noted above, "Pol" includes, but is not limited to, the
 30 protein-encoding regions comprising polymerase, protease, reverse transcriptase
 and/or integrase-containing sequences (Wan et al (1996) *Biochem. J.* 316:569-573;

Kohl et al. (1988) *PNAS USA* 85:4686-4690; Krausslich et al. (1988) *J. Virol.* 62:4393-4397; Coffin, "Retroviridae and their Replication" in *Virology*, pp1437-1500 (Raven, New York, 1990); Patel et. al. (1995) *Biochemistry* 34:5351-5363). Thus, the synthetic expression cassettes exemplified herein include one or more of these regions and one or more changes to the resulting amino acid sequences. Some exemplary polynucleotide sequences encoding Pol-derived polypeptides are presented in Table C.

The codon usage pattern for Pol was modified as described above for Gag and Env so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes.

Constructs may be modified in various ways. For example, the expression constructs may include a sequence that encodes the first 6 amino acids of the integrase polypeptide. This 6 amino acid region is believed to provide a cleavage recognition site recognized by HIV protease (see, e.g., McCornack et al. (1997) *FEBS Letts* 414:84-88). Constructs may include a multiple cloning site (MCS) for insertion of one or more transgenes, typically at the 3' end of the construct. In addition, a cassette encoding a catalytic center epitope derived from the catalytic center in RT is typically included 3' of the sequence encoding 6 amino acids of integrase. This cassette encodes Ile178 through Serine 191 of RT and may be added to keep this well conserved region as a possible CTL epitope. Further, the constructs contain an insertion mutations to preserve the reading frame. (see, e.g., Park et al. (1991) *J. Virol.* 65:5111).

In certain embodiments, the catalytic center and/or primer grip region of RT are modified. The catalytic center and primer grip regions of RT are described, for example, in Patel et al. (1995) *Biochem.* 34:5351 and Palaniappan et al. (1997) *J. Biol. Chem.* 272(17):11157. For example, wild type sequence encoding the amino acids YMDD at positions 183-185 of p66 RT, numbered relative to AF110975, may be replaced with sequence encoding the amino acids "AP". Further, the primer grip region (amino acids WMGY, residues 229-232 of p66RT, numbered relative to AF110975) may be replaced with sequence encoding the amino acids "PI."

For the Pol sequence, the changes in codon usage are typically restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame; however, regions within the frameshift translation region can be modified as well.

Finally, inhibitory (or instability) elements (INS) located within the coding sequences of the protease polypeptide coding sequence can be altered as well.

Experiments can be performed in support of the present invention to show that the synthetic Pol sequences were capable of higher level of protein production relative to the native Pol sequences. Modification of the Pol polypeptide coding sequences results in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Similar Pol polypeptide coding sequences can be obtained, modified and tested for improved expression from a variety of isolates, including those described above for Gag and Env.

The present invention also includes expression cassettes which include synthetic sequences derived HIV genes other than Gag, Env and Pol, including but not limited to, regions within Gag, Env, Pol, as well as, GagComplPolmut_C, GagComplPolmutAtt_C, GagComplPolmutIna_C, GagComplPolmutInaTatRevNef_C, GagPolmut_C, GagPolmutAtt_C, GagPolmutIna_C, GagProtInaRTmut_C, GagProtInaRTmutTatRevNef_C, GagRTmut_C, GagRTmutTatRevNef_C, GagTatRevNef_C, gp120mod.TV1.del118-210, gp120mod.TV1.delV1V2, gp120mod.TV1.delV2, gp140mod.TV1.del118-210, gp140mod.TV1.delV1V2, gp140mod.TV1.delV2, gp140mod.TV1.mut7, gp140mod.TV1.tpa2, gp140TMmod.TV1, gp160mod.TV1.del118-210, gp160mod.TV1.delV1V2, gp160mod.TV1.delV2, gp160mod.TV1.dV1, gp160mod.TV1.dV1-gagmod.BW965, gp160mod.TV1.dV1V2-gagmod.BW965, gp160mod.TV1.dV2-gagmod.BW965, gp160mod.TV1.tpa2, gp160mod.TV1-gagmod.BW965, int.opt.mut_C, int.opt_C, nef.D106G.-myr19.opt_C, p15RnaseH.opt_C, p2Pol.opt.YMWM_C, p2Polopt.YM_C, p2Polopt_C, p2PolTatRevNef opt C, p2PolTatRevNef.opt.native_C, p2PolTatRevNef.opt_C, protInaRT.YM.opt_C, protInaRT.YMWM.opt_C, ProtRT.TatRevNef.opt_C, rev.exon1_2.M5-10.opt_C, tat.exon1_2.opt.C22-37_C, tat.exon1_2.opt.C37_C, TatRevNef.opt.native_ZA, TatRevNef.opt_ZA, TatRevNefGag C, TatRevNefgagCpolIna C, TatRevNefGagProtInaRTmut C, and TatRevNefProtRT opt C. Sequences obtained from other strains can be manipulated in similar fashion following the teachings of the

present specification. As noted above, the codon usage pattern is modified as described above for Gag, Env and Pol so that the resulting nucleic acid coding sequence is comparable to codon usage found in highly expressed human genes. Typically these synthetic sequences are capable of higher level of protein production relative to the native sequences and that modification of the wild-type polypeptide coding sequences results in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Furthermore, the nucleic acid sequence can also be modified to introduce mutations into one or more regions of the gene, for instance to alter the function of the gene product (e.g., render the gene product non-functional) and/or to eliminate site modifications (e.g., the myristoylation site in Nef).

Synthetic expression cassettes, derived from HIV Type C coding sequences, exemplified herein include, but are not limited to, those comprising one or more of the following synthetic polynucleotides: GagComplPolmut_C, GagComplPolmutAtt_C, GagComplPolmutIna_C, GagComplPolmutInaTatRevNef_C, GagPolmut_C, GagPolmutAtt_C, GagPolmutIna_C, GagProtInaRTmut_C, GagProtInaRTmutTatRevNef_C, GagRTmut_C, GagRTmutTatRevNef_C, GagTatRevNef_C, gp120mod.TV1.del118-210, gp120mod.TV1.delV1V2, gp120mod.TV1.delV2, gp140mod.TV1.del118-210, gp140mod.TV1.delV1V2, gp140mod.TV1.delV2, gp140mod.TV1.mut7, gp140mod.TV1.tpa2, gp140TMmod.TV1, gp160mod.TV1.del118-210, gp160mod.TV1.delV1V2, gp160mod.TV1.delV2, gp160mod.TV1.dV1, gp160mod.TV1.dV1-gagmod.BW965, gp160mod.TV1.dV1V2-gagmod.BW965, gp160mod.TV1.dV2-gagmod.BW965, gp160mod.TV1.tpa2, gp160mod.TV1-gagmod.BW965, int.opt.mut_C, int.opt_C, nef.D106G.-myr19.opt_C, p15RnaseH.opt_C, p2Pol.opt.YMWM_C, p2Polopt.YM_C, p2Polopt_C, p2PolTatRevNef opt C, p2PolTatRevNef.opt.native_C, p2PolTatRevNef.opt_C, protInaRT.YM.opt_C, protInaRT.YMWM.opt_C, ProtRT.TatRevNef.opt_C, rev.exon1_2.M5-10.opt_C, tat.exon1_2.opt.C22-37_C, tat.exon1_2.opt.C37_C, TatRevNef.opt.native_ZA, TatRevNef.opt_ZA, TatRevNefGag C, TatRevNefgagCpollna C, TatRevNefGagProtInaRTmut C, and TatRevNefProtRT opt C.

Gag-complete refers to in-frame polyproteins comprising, e.g., Gag and pol, wherein the p6 portion of Gag is present.

Additional sequences that may be employed in some aspects of the present invention have been described in WO 00/39302, WO 00/39303, WO 00/39304, and
5 WO 02/04493.

2.2.2 FURTHER MODIFICATION OF SEQUENCES INCLUDING HIV NUCLEIC ACID CODING SEQUENCES

The HIV polypeptide-encoding expression cassettes described herein may also
10 contain one or more further sequences encoding, for example, one or more transgenes. Further sequences (*e.g.*, transgenes) useful in the practice of the present invention include, but are not limited to, further sequences are those encoding further viral epitopes/antigens {including but not limited to, HCV antigens (*e.g.*, E1, E2; Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton,
15 M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997), HIV antigens (*e.g.*, derived from one or more HIV
20 isolate); and sequences encoding tumor antigens/epitopes. Further sequences may also be derived from non-viral sources, for instance, sequences encoding cytokines such as interleukin-2 (IL-2), stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 12 (IL-12), G-CSF, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-1 alpha (IL-1 α), interleukin-11 (IL-11), MIP-1 α , tumor necrosis
25 factor (TNF), leukemia inhibitory factor (LIF), c-kit ligand, thrombopoietin (TPO) and flt3 ligand, commercially available from several vendors such as, for example, Genzyme (Framingham, MA), Genentech (South San Francisco, CA), Amgen (Thousand Oaks, CA), R&D Systems and Immunex (Seattle, WA). Additional sequences are described below. Also, variations on the orientation of the Gag and
30 other coding sequences, relative to each other, are described below.

HIV polypeptide coding sequences can be obtained from other HIV isolates, see, e.g., Myers et al. Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory. Synthetic expression
5 cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification.

Further, the synthetic expression cassettes of the present invention include related polypeptide sequences having greater than 85%, preferably greater than 90%, more preferably greater than 95%, and most preferably greater than 98% sequence
10 identity to the polypeptides encoded by the synthetic expression cassette sequences disclosed herein.

Exemplary expression cassettes and modifications are set forth in Example 1.

2.2.3 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1

15 POLYPEPTIDES AND RELATED POLYPEPTIDES

Synthetic HIV-encoding sequences (expression cassettes) of the present invention can be cloned into a number of different expression vectors to evaluate levels of expression and, in the case of Gag-containing constructs, production of VLPs. The synthetic DNA fragments for HIV polypeptides can be cloned into eucaryotic
20 expression vectors, including, a transient expression vector, CMV-promoter-based mammalian vectors, and a shuttle vector for use in baculovirus expression systems. Corresponding wild-type sequences can also be cloned into the same vectors.

These vectors can then be transfected into a several different cell types, including a variety of mammalian cell lines (293, RD, COS-7, and CHO, cell lines
25 available, for example, from the A.T.C.C.). The cell lines are then cultured under appropriate conditions and the levels of any appropriate polypeptide product can be evaluated in supernatants. (see, Table A). For example, p24 can be used to evaluate Gag expression; gp160, gp140 or gp120 can be used to evaluate Env expression; p6pol can be used to evaluate Pol expression; prot can be used to evaluate protease;
30 p15 for RNaseH; p31 for Integrase; and other appropriate polypeptides for Vif, Vpr, Tat, Rev, Vpu and Nef. Further, modified polypeptides can also be used, for example,

other Env polypeptides include, but are not limited to, for example, native gp160, oligomeric gp140, monomeric gp120 as well as modified and/or synthetic sequences of these polypeptides. The results of these assays demonstrate that expression of synthetic HIV polypeptide-encoding sequences are significantly higher than
5 corresponding wild-type sequences.

Further, Western Blot analysis can be used to show that cells containing the synthetic expression cassette produce the expected protein at higher per-cell concentrations than cells containing the native expression cassette. The HIV proteins can be seen in both cell lysates and supernatants. The levels of production are
10 significantly higher in cell supernatants for cells transfected with the synthetic expression cassettes of the present invention.

Fractionation of the supernatants from mammalian cells transfected with the synthetic expression cassette can be used to show that the cassettes provide superior production of HIV proteins and, in the case of Gag, VLPs, relative to the wild-type
15 sequences.

Efficient expression of these HIV-containing polypeptides in mammalian cell lines provides the following benefits: the polypeptides are free of baculovirus contaminants; production by established methods approved by the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of
20 producing the Sub HIV-containing polypeptides in CHO cells which is not feasible in the absence of the increased expression obtained using the constructs of the present invention. Exemplary Mammalian cell lines include, but are not limited to, BHK, VERO, HT1080, 293, 293T, RD, COS-7, CHO, Jurkat, HUT, SUPT, C8166, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, and CEMX174 (such cell lines are
25 available, for example, from the A.T.C.C.).

A synthetic Gag expression cassette of the present invention will also exhibit high levels of expression and VLP production when transfected into insect cells. Synthetic expression cassettes described herein also demonstrate high levels of expression in insect cells. Further, in addition to a higher total protein yield, the final
30 product from the synthetic polypeptides consistently contains lower amounts of contaminating baculovirus proteins than the final product from the native sequences.

Further, synthetic expression cassettes of the present invention can also be introduced into yeast vectors which, in turn, can be transformed into and efficiently expressed by yeast cells (*Saccharomyces cerevisiae*; using vectors as described in Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998).

In addition to the mammalian and insect vectors, the synthetic expression cassettes of the present invention can be incorporated into a variety of expression vectors using selected expression control elements. Appropriate vectors and control elements for any given cell can be selected by one having ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors.

For example, a synthetic expression cassette can be inserted into a vector which includes control elements operably linked to the desired coding sequence, which allow for the expression of the gene in a selected cell-type. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter (a CMV promoter can include intron A), RSV, HIV-Ltr, the mouse mammary tumor virus LTR promoter (MMLV-ltr), the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook, et al., *supra*, as well as a bovine growth hormone terminator sequence. Introns, containing splice donor and acceptor sites, may also be designed into the constructs for use with the present invention (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the

long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

5 The desired synthetic polypeptide encoding sequences can be cloned into any number of commercially available vectors to generate expression of the polypeptide in an appropriate host system. These systems include, but are not limited to, the following: baculovirus expression {Reilly, P.R., et al., BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL (1992); Beames, et al., *Biotechniques* 11:378
10 (1991); Pharmingen; Clontech, Palo Alto, CA)}, vaccinia expression {Earl, P. L., et al., "Expression of proteins in mammalian cells using vaccinia" In *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. Eds.), Greene Publishing Associates & Wiley Interscience, New York (1991); Moss, B., et al., U.S. Patent Number 5,135,855, issued 4 August 1992}, expression in bacteria {Ausubel, F.M., et al.,
15 CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA; Clontech}, expression in yeast {Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998; Shuster, J.R., U.S. Patent No. 5,629,203, issued May 13, 1997; Gellissen, G., et al., *Antonie Van Leeuwenhoek*, 62(1-2):79-93 (1992); Romanos, M.A., et al., *Yeast* 8(6):423-488 (1992); Goeddel, D.V., *Methods in Enzymology* 185 (1990); Guthrie, C., and G.R. Fink, *Methods in Enzymology* 194
20 (1991)}, expression in mammalian cells {Clontech; Gibco-BRL, Ground Island, NY; e.g., Chinese hamster ovary (CHO) cell lines (Haynes, J., et al., *Nuc. Acid. Res.* 11:687-706 (1983); 1983, Lau, Y.F., et al., *Mol. Cell. Biol.* 4:1469-1475 (1984); Kaufman, R. J., "Selection and coamplification of heterologous genes in mammalian
25 cells," in *Methods in Enzymology*, vol. 185, pp537-566. Academic Press, Inc., San Diego CA (1991)}, and expression in plant cells {plant cloning vectors, Clontech Laboratories, Inc., Palo Alto, CA, and Pharmacia LKB Biotechnology, Inc., Piscataway, NJ; Hood, E., et al., *J. Bacteriol.* 168:1291-1301 (1986); Nagel, R., et al., *FEMS Microbiol. Lett.* 67:325 (1990); An, et al., "Binary Vectors", and others in
30 Plant Molecular Biology Manual A3:1-19 (1988); Miki, B.L.A., et al., pp.249-265, and others in Plant DNA Infectious Agents (Hohn, T., et al., eds.) Springer-Verlag,

Wien, Austria, (1987); *Plant Molecular Biology: Essential Techniques*, P.G. Jones and J.M. Sutton, New York, J. Wiley, 1997; Miglani, Gurbachan *Dictionary of Plant Genetics and Molecular Biology*, New York, Food Products Press, 1998; Henry, R. J., *Practical Applications of Plant Molecular Biology*, New York, Chapman & Hall, 5 1997}.

Also included in the invention is an expression vector, containing coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation codon, and translation and transcription termination sequences, 10 and an insertion site for introducing the insert into the vector. Translational control elements have been reviewed by M. Kozak (e.g., Kozak, M., *Mamm. Genome* 7(8):563-574, 1996; Kozak, M., *Biochimie* 76(9):815-821, 1994; Kozak, M., *J Cell Biol* 108(2):229-241, 1989; Kozak, M., and Shatkin, A.J., *Methods Enzymol* 60:360-375, 1979).

15 Expression in yeast systems has the advantage of commercial production. Recombinant protein production by vaccinia and CHO cell line have the advantage of being mammalian expression systems. Further, vaccinia virus expression has several advantages including the following: (i) its wide host range; (ii) faithful post-transcriptional modification, processing, folding, transport, secretion, and assembly of 20 recombinant proteins; (iii) high level expression of relatively soluble recombinant proteins; and (iv) a large capacity to accommodate foreign DNA.

The recombinantly expressed polypeptides from synthetic HIV polypeptide-encoding expression cassettes are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt 25 fractionation, ion exchange chromatography, gel filtration, size-exclusion chromatography, size-fractionation, and affinity chromatography. Immunoaffinity chromatography can be employed using antibodies generated based on, for example, HIV antigens.

Advantages of expressing the proteins of the present invention using 30 mammalian cells include, but are not limited to, the following: well-established protocols for scale-up production; the ability to produce VLPs; cell lines are suitable to

meet good manufacturing process (GMP) standards; culture conditions for mammalian cells are known in the art.

Synthetic HIV 1 polynucleotides are described herein, see, for example, the figures. Various forms of the different embodiments of the invention, described herein,
5 may be combined.

Exemplary expression assays are set forth in Example 2. Exemplary conditions for Western Blot analysis are presented in Example 3.

10 2.3.0 PRODUCTION OF VIRUS-LIKE PARTICLES AND USE OF THE CONSTRUCTS OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES.

The group-specific antigens (Gag) of human immunodeficiency virus type-1 (HIV-1) self-assemble into noninfectious virus-like particles (VLP) that are released from various eucaryotic cells by budding (reviewed by Freed, E.O., *Virology* **251**:1-15,
15 1998). The Gag-containing synthetic expression cassettes of the present invention provide for the production of HIV-Gag virus-like particles (VLPs) using a variety of different cell types, including, but not limited to, mammalian cells.

Viral particles can be used as a matrix for the proper presentation of an antigen entrapped or associated therewith to the immune system of the host.

20

2.3.1 VLP PRODUCTION USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

The Gag-containing synthetic expression cassettes of the present invention may provide superior production of both Gag proteins and VLPs, relative to native Gag
25 coding sequences. Further, electron microscopic evaluation of VLP production can be used to show that free and budding immature virus particles of the expected size are produced by cells containing the synthetic expression cassettes.

Using the synthetic expression cassettes of the present invention, rather than native Gag coding sequences, for the production of virus-like particles provide several
30 advantages. First, VLPs can be produced in enhanced quantity making isolation and purification of the VLPs easier. Second, VLPs can be produced in a variety of cell

types using the synthetic expression cassettes, in particular, mammalian cell lines can be used for VLP production, for example, CHO cells. Production using CHO cells provides (i) VLP formation; (ii) correct myristoylation and budding; (iii) absence of non-mamallian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of
5 purification. The synthetic expression cassettes of the present invention are also useful for enhanced expression in cell-types other than mammalian cell lines. For example, infection of insect cells with baculovirus vectors encoding the synthetic expression cassettes results in higher levels of total Gag protein yield and higher levels of VLP production (relative to wild-oding sequences). Further, the final product from insect
10 cells infected with the baculovirus-Gag synthetic expression cassettes consistently contains lower amounts of contaminating insect proteins than the final product when wild-oding sequences are used.

VLPs can spontaneously form when the particle-forming polypeptide of
15 interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs produced using the synthetic expression cassettes of the present invention are conveniently prepared using recombinant techniques. As discussed below, the Gag polypeptide encoding synthetic expression cassettes of the present invention can include other polypeptide coding sequences of interest (for example, HIV protease,
20 HIV polymerase, Env; synthetic Env). Expression of such synthetic expression cassettes yields VLPs comprising the Gag polypeptide, as well as, the polypeptide of interest.

Once coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be cloned into any suitable vector or replicon for
25 expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Sambrook et al, *supra*. The vector is then used to transform an appropriate host cell. Suitable recombinant expression systems include, but are not limited to, bacterial, mammalian, baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses
30 (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian, yeast and Xenopus expression systems, well known in the art. Particularly preferred expression

systems are mammalian cell lines, vaccinia, Sindbis, eucaryotic layered vector initiation systems (e.g., US Patent No. 6,015,686, US Patent No. 5, 814,482, US Patent No. 6,015,694, US Patent No. 5,789,245, EP 1029068A2, WO 9918226A2/A3, EP 00907746A2, WO 9738087A2), insect and yeast systems.

5 The synthetic DNA fragments for the expression cassettes of the present invention, e.g., Pol, Gag, Env, Tat, Rev, Nef, Vif, Vpr, and/or Vpu, may be cloned into the following eucaryotic expression vectors: pCMVKm2, for transient expression assays and DNA immunization studies, the pCMVKm2 vector is derived from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) and comprises a
10 kanamycin selectable marker, a ColE1 origin of replication, a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a polylinker site is inserted into pCMVKm2 to generate pCMV-link; pESN2dhfr and pCMVPLEdhfr, for
15 expression in Chinese Hamster Ovary (CHO) cells; and, pAcC13, a shuttle vector for use in the Baculovirus expression system (pAcC13, is derived from pAcC12 which is described by Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990).

Briefly, construction of pCMVPLEdhfr was as follows.

To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site)
20 leader was PCR-amplified from pCite-4a+ (Novagen, Inc., Milwaukee, WI) and inserted into pET-23d (Novagen, Inc., Milwaukee, WI) as an *Xba*-*Nco* fragment to give pET-EMCV. The *dhfr* gene was PCR-amplified from pESN2dhfr to give a product with a Gly-Gly-Gly-Ser spacer in place of the translation stop codon and inserted as an *Nco*-*Bam*H1 fragment to give pET-E-DHFR. Next, the attenuated *neo*
25 gene was PCR amplified from a pSV2Neo (Clontech, Palo Alto, CA) derivative and inserted into the unique *Bam*H1 site of pET-E-DHFR to give pET-E-DHFR/Neo_(m2). Finally the bovine growth hormone terminator from pCDNA3 (Invitrogen, Inc., Carlsbad, CA) was inserted downstream of the *neo* gene to give pET-E-DHFR/Neo_(m2)BGHt. The EMCV-*dhfr/neo* selectable marker cassette fragment was
30 prepared by cleavage of pET-E-DHFR/Neo_(m2)BGHt.

In one vector construct the CMV enhancer/promoter plus Intron A was transferred from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) as a *HindIII-SalI* fragment into pUC19 (New England Biolabs, Inc., Beverly, MA). The vector backbone of pUC19 was deleted from the *NdeI* to the *SapI* sites. The above
5 described DHFR cassette was added to the construct such that the EMCV IRES followed the CMV promoter. The vector also contained an *amp^r* gene and an SV40 origin of replication.

A number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (A.T.C.C.), such
10 as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula*
15 *polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guilliermondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*. See, e.g., Summers and Smith, *Texas Agricultural Experiment Station*
20 *Bulletin No. 1555* (1987).

Viral vectors can be used for the production of particles in eucaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additionally, a vaccinia based infection/transfection system, as described in Tomei et al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993)
25 74:1103-1113, will also find use with the present invention. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the
30 cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery.

Alternately, T7 can be added as a purified protein or enzyme as in the "Progenitor" system (Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130). The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

5 Depending on the expression system and host selected, the VLPS are produced by growing host cells transformed by an expression vector under conditions whereby the particle-forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPs are formed intracellularly, the cells are then disrupted, using chemical, physical or
10 mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

 The particles are then isolated (or substantially purified) using methods that
15 preserve the integrity thereof, such as, by gradient centrifugation, e.g., cesium chloride (CsCl) sucrose gradients, pelleting and the like (see, e.g., Kirnbauer et al. *J. Virol.* (1993) 67:6929-6936), as well as standard purification techniques including, e.g., ion exchange and gel filtration chromatography.

 VLPs produced by cells containing the synthetic expression cassettes of the
20 present invention can be used to elicit an immune response when administered to a subject. One advantage of the present invention is that VLPs can be produced by mammalian cells carrying the synthetic expression cassettes at levels previously not possible. As discussed above, the VLPs can comprise a variety of antigens in addition to the Gag polypeptide (e.g., Gag-protease, Gag-polymerase, Env, synthetic Env,
25 etc.). Purified VLPs, produced using the synthetic expression cassettes of the present invention, can be administered to a vertebrate subject, usually in the form of vaccine compositions. Combination vaccines may also be used, where such vaccines contain, for example, an adjuvant subunit protein (e.g., Env). Administration can take place using the VLPs formulated alone or formulated with other antigens. Further, the
30 VLPs can be administered prior to, concurrent with, or subsequent to, delivery of the synthetic expression cassettes for DNA immunization (see below) and/or delivery of

other vaccines. Also, the site of VLP administration may be the same or different as other vaccine compositions that are being administered. Gene delivery can be accomplished by a number of methods including, but are not limited to, immunization with DNA, alphavirus vectors, pox virus vectors, and vaccinia virus vectors.

5 VLP immune-stimulating (or vaccine) compositions can include various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. The immune stimulating compositions will include an amount of the VLP/antigen sufficient to mount an immunological response. An appropriate effective amount can be determined by one of skill in the art. Such an amount will fall in a relatively broad
10 range that can be determined through routine trials and will generally be an amount on the order of about 0.1 μ g to about 1000 μ g, more preferably about 1 μ g to about 300 μ g, of VLP/antigen.

A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition.
15 Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from
20 poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated
25 to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from *E. coli*.

Adjuvants may also be used to enhance the effectiveness of the compositions. Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water
30 emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for

example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particle generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (6) oligonucleotides or polymeric molecules encoding immunostimulatory CpG motifs (Davis, H.L., et al., *J. Immunology* **160**:870-876, 1998; Sato, Y. et al., *Science* **273**:352-354, 1996) or complexes of antigens/oligonucleotides {Polymeric molecules include double and single stranded RNA and DNA, and backbone modifications thereof, for example, methylphosphonate linkages; or (7) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); and (8) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Further, such polymeric molecules include alternative polymer backbone structures such as, but not limited to, polyvinyl backbones (Pitha, *Biochem Biophys Acta*, **204**:39, 1970a;

Pitha, *Biopolymers*, 9:965, 1970b), and morpholino backbones (Summerton, J., *et al.*, U.S. Patent No. 5,142,047, issued 08/25/92; Summerton, J., *et al.*, U.S. Patent No. 5,185,444 issued 02/09/93). A variety of other charged and uncharged polynucleotide analogs have been reported. Numerous backbone modifications are known in the art, including, but not limited to, uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, and carbamates) and charged linkages (*e.g.*, phosphorothioates and phosphorodithioates).}; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the VLP immune-stimulating (or vaccine) composition. Alum, CpG oligonucleotides, and MF59 are preferred.

10 Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Dosage treatment with the VLP composition may be a single dose schedule or a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the subject and be dependent on the judgment of the practitioner.

20 If prevention of disease is desired, the antigen carrying VLPs are generally administered prior to primary infection with the pathogen of interest. If treatment is desired, *e.g.*, the reduction of symptoms or recurrences, the VLP compositions are generally administered subsequent to primary infection.

25

2.3.2 USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

A number of viral based systems have been developed for use as gene transfer vectors for mammalian host cells. For example, retroviruses (in particular, lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful for gene therapy applications) can be

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inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described, including, for example, the following: (U.S. Patent No. 5,219,740; Miller et al. (1989) *BioTechniques* 7:980; Miller, A.D. (1990) *Human Gene Therapy* 1:5; Scarpa et al. (1991) *Virology* 180:849; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033; Boris-Lawrie et al. (1993) *Cur. Opin. Genet. Develop.* 3:102; GB 2200651; EP 0415731; EP 0345242; WO 89/02468; WO 89/05349; WO 89/09271; WO 90/02806; WO 90/07936; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; in U.S. 5,219,740; U.S. 4,405,712; U.S. 4,861,719; U.S. 4,980,289 and U.S. 4,777,127; in U.S. Serial No. 07/800,921; and in Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53:83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci USA* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

In other embodiments, gene transfer vectors can be constructed to encode a cytokine or other immunomodulatory molecule. For example, nucleic acid sequences encoding native IL-2 and gamma-interferon can be obtained as described in US Patent Nos. 4,738,927 and 5,326,859, respectively, while useful muteins of these proteins can be obtained as described in U.S. Patent No. 4,853,332. Nucleic acid sequences encoding the short and long forms of mCSF can be obtained as described in US Patent Nos. 4,847,201 and 4,879,227, respectively. In particular aspects of the invention, retroviral vectors expressing cytokine or immunomodulatory genes can be produced as described herein (for example, employing the packaging cell lines of the present invention) and in International Application No. PCT US 94/02951, entitled "Compositions and Methods for Cancer Immunotherapy."

Examples of suitable immunomodulatory molecules for use herein include the following: IL-1 and IL-2 (Karupiah et al. (1990) *J. Immunology* 144:290-298, Weber et al. (1987) *J. Exp. Med.* 166:1716-1733, Gansbacher et al. (1990) *J. Exp. Med.* 172:1217-1224, and U.S. Patent No. 4,738,927); IL-3 and IL-4 (Tepper et al. (1989) *Cell* 57:503-512, Golumbek et al. (1991) *Science* 254:713-716, and U.S. Patent No.

5,017,691); IL-5 and IL-6 (Brakenhof et al. (1987) *J. Immunol.* 139:4116-4121, and International Publication No. WO 90/06370); IL-7 (U.S. Patent No. 4,965,195); IL-8, IL-9, IL-10, IL-11, IL-12, and IL-13 (*Cytokine Bulletin*, Summer 1994); IL-14 and IL-15; alpha interferon (Finter et al. (1991) *Drugs* 42:749-765, U.S. Patent Nos. 4,892,743 and 4,966,843, International Publication No. WO 85/02862, Nagata et al. (1980) *Nature* 284:316-320, Familletti et al. (1981) *Methods in Enz.* 78:387-394, Twu et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2046-2050, and Faktor et al. (1990) *Oncogene* 5:867-872); beta-interferon (Seif et al. (1991) *J. Virol.* 65:664-671); gamma-interferons (Radford et al. (1991) *The American Society of Hepatology* 20082015, Watanabe et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9456-9460, Gansbacher et al. (1990) *Cancer Research* 50:7820-7825, Maio et al. (1989) *Can. Immunol. Immunother.* 30:34-42, and U.S. Patent Nos. 4,762,791 and 4,727,138); G-CSF (U.S. Patent Nos. 4,999,291 and 4,810,643); GM-CSF (International Publication No. WO 85/04188).

15 Immunomodulatory factors may also be agonists, antagonists, or ligands for these molecules. For example, soluble forms of receptors can often behave as antagonists for these types of factors, as can mutated forms of the factors themselves.

Nucleic acid molecules that encode the above-described substances, as well as other nucleic acid molecules that are advantageous for use within the present invention, may be readily obtained from a variety of sources, including, for example, depositories such as the American Type Culture Collection, or from commercial sources such as British Bio-Technology Limited (Cowley, Oxford England). Representative examples include BBG 12 (containing the GM-CSF gene coding for the mature protein of 127 amino acids), BBG 6 (which contains sequences encoding gamma interferon), A.T.C.C. Deposit No. 39656 (which contains sequences encoding TNF), A.T.C.C. Deposit No. 20663 (which contains sequences encoding alpha-interferon), A.T.C.C. Deposit Nos. 31902, 31902 and 39517 (which contain sequences encoding beta-interferon), A.T.C.C. Deposit No. 67024 (which contains a sequence which encodes Interleukin-1b), A.T.C.C. Deposit Nos. 39405, 39452, 39516, 39626 and 39673 (which contain sequences encoding Interleukin-2), A.T.C.C. Deposit Nos. 59399, 59398, and 67326 (which contain sequences encoding Interleukin-3), A.T.C.C.

Deposit No. 57592 (which contains sequences encoding Interleukin-4), A.T.C.C. Deposit Nos. 59394 and 59395 (which contain sequences encoding Interleukin-5), and A.T.C.C. Deposit No. 67153 (which contains sequences encoding Interleukin-6).

5 Plasmids containing cytokine genes or immunomodulatory genes (International Publication Nos. WO 94/02951 and WO 96/21015) can be digested with appropriate restriction enzymes, and DNA fragments containing the particular gene of interest can be inserted into a gene transfer vector using standard molecular biology techniques. (See, e.g., Sambrook et al., *supra.*, or Ausbel et al. (eds) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience).

10 Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. For example, plasmids which contain sequences that encode altered cellular products may be obtained from a depository such as the A.T.C.C., or
15 from commercial sources. Plasmids containing the nucleotide sequences of interest can be digested with appropriate restriction enzymes, and DNA fragments containing the nucleotide sequences can be inserted into a gene transfer vector using standard molecular biology techniques.

Alternatively, cDNA sequences for use with the present invention may be
20 obtained from cells which express or contain the sequences, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra.*, for a description of techniques used to obtain and isolate DNA. Briefly, mRNA from a cell which expresses the gene of interest can be reverse transcribed with reverse transcriptase using oligo-dT or random primers. The single stranded cDNA
25 may then be amplified by PCR (see U.S. Patent Nos. 4,683,202, 4,683,195 and 4,800,159, see also *PCR Technology: Principles and Applications for DNA Amplification*, Erlich (ed.), Stockton Press, 1989)) using oligonucleotide primers complementary to sequences on either side of desired sequences.

The nucleotide sequence of interest can also be produced synthetically, rather
30 than cloned, using a DNA synthesizer (e.g., an Applied Biosystems Model 392 DNA Synthesizer, available from ABI, Foster City, California). The nucleotide sequence can

be designed with the appropriate codons for the expression product desired. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.

The synthetic expression cassettes of the present invention can be employed in the construction of packaging cell lines for use with retroviral vectors.

One type of retrovirus, the murine leukemia virus, or "MLV", has been widely utilized for gene therapy applications (see generally Mann et al. (*Cell* 33:153, 1993), Cane and Mulligan (*Proc. Nat'l. Acad. Sci. USA* 81:6349, 1984), and Miller et al., *Human Gene Therapy* 1:5-14, 1990).

Lentiviral vectors typically, comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3' lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5') or downstream (3') of a coding sequence of interest (for example, a synthetic Gag or Env expression cassette of the present invention). Within certain embodiments, the nuclear transport element is not RRE. Within one embodiment the packaging signal is an extended packaging signal. Within other embodiments the promoter is a tissue specific promoter, or, alternatively, a promoter such as CMV. Within other embodiments, the lentiviral vector further comprises an internal ribosome entry site.

A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV and SIV.

Within yet another aspect of the invention, host cells (e.g., packaging cell lines) are provided which contain any of the expression cassettes described herein. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Gag-polymerase. Packaging cell lines may further comprise a promoter and a sequence

encoding tat, rev, or an envelope, wherein the promoter is operably linked to the sequence encoding tat, rev, Env or sequences encoding modified versions of these proteins. The packaging cell line may further comprise a sequence encoding any one or more of other HIV gene encoding sequences.

5 In one embodiment, the expression cassette (carrying, for example, the synthetic Gag-polymerase) is stably integrated. The packaging cell line, upon introduction of a lentiviral vector, typically produces particles. The promoter regulating expression of the synthetic expression cassette may be inducible. Typically, the packaging cell line, upon introduction of a lentiviral vector, produces particles that
10 are essentially free of replication competent virus.

 Packaging cell lines are provided comprising an expression cassette which directs the expression of a synthetic *Gag-polymerase* gene or comprising an expression cassette which directs the expression of a synthetic Env genes described herein. (See, also, Andre, S., et al., *Journal of Virology* 72(2):1497-1503, 1998; Haas, J., et al.,
15 *Current Biology* 6(3):315-324, 1996) for a description of other modified Env sequences). A lentiviral vector is introduced into the packaging cell line to produce a vector producing cell line.

 As noted above, lentiviral vectors can be designed to carry or express a selected gene(s) or sequences of interest. Lentiviral vectors may be readily
20 constructed from a wide variety of lentiviruses (*see* RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Representative examples of lentiviruses included HIV, HIV-1, HIV-2, FIV and SIV. Such lentiviruses may either be obtained from patient isolates, or, more preferably, from depositories or collections such as the American Type Culture Collection, or isolated from known sources using
25 available techniques.

 Portions of the lentiviral gene delivery vectors (or vehicles) may be derived from different viruses. For example, in a given recombinant lentiviral vector, LTRs may be derived from an HIV, a packaging signal from SIV, and an origin of second strand synthesis from HrV-2. Lentiviral vector constructs may comprise a 5' lentiviral
30 LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences,

an origin of second strand DNA synthesis and a 3' LTR, wherein said lentiviral vector contains a nuclear transport element that is not RRE.

Briefly, Long Terminal Repeats ("LTRs") are subdivided into three elements, designated U5, R and U3. These elements contain a variety of signals which are responsible for the biological activity of a retrovirus, including for example, promoter and enhancer elements which are located within U3. LTRs may be readily identified in the provirus (integrated DNA form) due to their precise duplication at either end of the genome. As utilized herein, a 5' LTR should be understood to include a 5' promoter element and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector. The 3' LTR should be understood to include a polyadenylation signal, and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector.

The tRNA binding site and origin of second strand DNA synthesis are also important for a retrovirus to be biologically active, and may be readily identified by one of skill in the art. For example, retroviral tRNA binds to a tRNA binding site by Watson-Crick base pairing, and is carried with the retrovirus genome into a viral particle. The tRNA is then utilized as a primer for DNA synthesis by reverse transcriptase. The tRNA binding site may be readily identified based upon its location just downstream from the 5'LTR. Similarly, the origin of second strand DNA synthesis is, as its name implies, important for the second strand DNA synthesis of a retrovirus. This region, which is also referred to as the poly-purine tract, is located just upstream of the 3'LTR.

In addition to a 5' and 3' LTR, tRNA binding site, and origin of second strand DNA synthesis, recombinant retroviral vector constructs may also comprise a packaging signal, as well as one or more genes or coding sequences of interest. In addition, the lentiviral vectors have a nuclear transport element which, in preferred embodiments is not RRE. Representative examples of suitable nuclear transport elements include the element in Rous sarcoma virus (Ogert, et al., *J ViroL* 70, 3834-3843, 1996), the element in Rous sarcoma virus (Liu & Mertz, *Genes & Dev.*, 9, 1766-1789, 1995) and the element in the genome of simian retrovirus type I (Zolotukhin, et al., *J Virol.* 68, 7944-7952, 1994). Other potential elements include the elements in

the histone gene (Kedes, *Annu. Rev. Biochem.* 48, 837-870, 1970), the α -interferon gene (Nagata et al., *Nature* 287, 401-408, 1980), the β -adrenergic receptor gene (Koilkka, et al., *Nature* 329, 75-79, 1987), and the c-Jun gene (Hattorie, et al., *Proc. Natl. Acad. Sci. USA* 85, 9148-9152, 1988).

5 Recombinant lentiviral vector constructs typically lack both *Gag-polymerase* and *Env* coding sequences. Recombinant lentiviral vector typically contain less than 20, preferably 15, more preferably 10, and most preferably 8 consecutive nucleotides found in *Gag-polymerase* and *Env* genes. One advantage of the present invention is that the synthetic *Gag-polymerase* expression cassettes, which can be used to
10 construct packaging cell lines for the recombinant retroviral vector constructs, have little homology to wild-type *Gag-polymerase* sequences and thus considerably reduce or eliminate the possibility of homologous recombination between the synthetic and wild-type sequences.

 Lentiviral vectors may also include tissue-specific promoters to drive
15 expression of one or more genes or sequences of interest.

 Lentiviral vector constructs may be generated such that more than one gene of interest is expressed. This may be accomplished through the use of di- or oligo-cistronic cassettes (e.g., where the coding regions are separated by 80 nucleotides or less, *see generally* Levin et al., *Gene* 108:167-174, 1991), or through the use of
20 Internal Ribosome Entry Sites ("IRES").

 Packaging cell lines suitable for use with the above described recombinant retroviral vector constructs may be readily prepared given the disclosure provided herein. Briefly, the parent cell line from which the packaging cell line is derived can be selected from a variety of mammalian cell lines, including for example, 293, RD, COS-
25 7, CHO, BHK, VERO, HT1080, and myeloma cells.

 After selection of a suitable host cell for the generation of a packaging cell line, one or more expression cassettes are introduced into the cell line in order to complement or supply in *trans* components of the vector which have been deleted.

 Representative examples of suitable synthetic HIV polynucleotide sequences
30 have been described herein for use in expression cassettes of the present invention. As

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described above, the native and/or synthetic coding sequences may also be utilized in these expression cassettes.

Utilizing the above-described expression cassettes, a wide variety of packaging cell lines can be generated. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Gag-polymerase. Within other aspects, packaging cell lines are provided comprising a promoter and a sequence encoding tat, rev, Env, or other HIV antigens or epitopes derived therefrom, wherein the promoter is operably linked to the sequence encoding tat, rev, Env, or the HIV antigen or epitope. Within further embodiments, the packaging cell line may comprise a sequence encoding any one or more of tat, rev, nef, vif, vpu or vpr. For example, the packaging cell line may contain only tat, rev, nef, vif, vpu, or vpr alone, tat rev and nef, nef and vif, nef and vpu, nef and vpr, vif and vpu, vif and vpr, vpu and vpr, nef vif and vpu, nef vif and vpr, nef vpu and vpr, vif vpu and vpr, all four of nef, vif, vpu, and vpr, etc.

In one embodiment, the expression cassette is stably integrated. Within another embodiment, the packaging cell line, upon introduction of a lentiviral vector, produces particles. Within further embodiments the promoter is inducible. Within certain preferred embodiments of the invention, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are free of replication competent virus.

The synthetic cassettes containing modified coding sequences are transfected into a selected cell line. Transfected cells are selected that (i) carry, typically, integrated, stable copies of the HIV coding sequences, and (ii) are expressing acceptable levels of these polypeptides (expression can be evaluated by methods known in the prior art in view of the teachings of the present disclosure). The ability of the cell line to produce VLPs may also be verified.

A sequence of interest is constructed into a suitable viral vector as discussed above. This defective virus is then transfected into the packaging cell line. The packaging cell line provides the viral functions necessary for producing virus-like particles into which the defective viral genome, containing the sequence of interest, are

packaged. These VLPs are then isolated and can be used, for example, in gene delivery or gene therapy.

Further, such packaging cell lines can also be used to produce VLPs alone, which can, for example, be used as adjuvants for administration with other antigens or in vaccine compositions. Also, co-expression of a selected sequence of interest encoding a polypeptide (for example, an antigen) in the packaging cell line can also result in the entrapment and/or association of the selected polypeptide in/with the VLPs.

Various forms of the different embodiments of the present invention (*e.g.*, synthetic constructs) may be combined.

2.4.0 DNA IMMUNIZATION AND GENE DELIVERY

A variety of HIV polypeptide antigens, particularly HIV antigens, can be used in the practice of the present invention. HIV antigens can be included in DNA immunization constructs containing, for example, a synthetic Env expression cassettes, a synthetic Gag expression cassette, a synthetic pol-derived polypeptide expression cassette, a synthetic expression cassette comprising sequences encoding one or more accessory or regulatory genes (*e.g.*, tat, rev, nef, vif, vpu, vpr), and/or a synthetic Gag expression cassette fused in-frame to a coding sequence for the polypeptide antigen (synthetic or wild-type), where expression of the construct results in VLPs presenting the antigen of interest.

HIV antigens of particular interest to be used in the practice of the present invention include pol, tat, rev, nef, vif, vpu, vpr, and other HIV-1 (also known as HTLV-III, LAV, ARV, etc.) antigens or epitopes derived therefrom, including, but not limited to, antigens such as gp120, gp41, gp160 (both native and modified); Gag; and pol from a variety of isolates including, but not limited to, HIV_{IIIb}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (*e.g.*, subtypes, A through G, and O), HIV-2 strains and diverse subtypes (*e.g.*, HIV-2_{UC1} and HIV-2_{UC2}). See, *e.g.*, Myers, et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico; Myers, et al.,

Human Retroviruses and Aids, 1990, Los Alamos, New Mexico: Los Alamos National Laboratory. These antigens may be synthetic (as described herein) or wild-type.

To evaluate efficacy, DNA immunization using synthetic expression cassettes of the present invention can be performed, for example, as follows. Mice are
5 immunized with a tat/rev/nef synthetic expression cassette. Other mice are immunized with a tat/rev/nef wild type expression cassette. Mouse immunizations with plasmid-DNAs typically show that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, a second boost immunization will induce a secondary immune response, for example,
10 after approximately two weeks. Further, the results of CTL assays typically show increased potency of synthetic expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

Exemplary primate studies directed at the evaluation of neutralizing antibodies and cellular immune responses against HIV are described below.

15 It is readily apparent that the subject invention can be used to mount an immune response to a wide variety of antigens and hence to treat or prevent infection, particularly HIV infection.

2.4.1 DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE 20 PRESENT INVENTION

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. Furthermore, the desired gene can be isolated directly from cells
25 and tissues containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired.
30 In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence is assembled from overlapping

oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene* 164:49-53.

- 5 Next, the gene sequence encoding the desired antigen can be inserted into a vector containing a synthetic expression cassette of the present invention. In one embodiment, polynucleotides encoding selected antigens are separately cloned into expression vectors (e.g., Env-coding polynucleotide in a first vector, Gag-coding polynucleotide in a second vector, Pol-derived polypeptide-coding polynucleotide in a third vector, tat-, rev-, nef-, vif-, vpu-, vpr-coding polynucleotides in further vectors, etc.). In certain embodiments, the antigen is inserted into or adjacent a synthetic Gag coding sequence such that when the combined sequence is expressed it results in the production of VLPs comprising the Gag polypeptide and the antigen of interest, e.g., Env (native or modified) or other antigen(s) (native or modified) derived from HIV.
- 10 Insertions can be made within the coding sequence or at either end of the coding sequence (5', amino terminus of the expressed Gag polypeptide; or 3', carboxy terminus of the expressed Gag polypeptide)(Wagner, R., et al., *Arch Virol.* 127:117-137, 1992; Wagner, R., et al., *Virology* 200:162-175, 1994; Wu, X., et al., *J. Virol.* 69(6):3389-3398, 1995; Wang, C-T., et al., *Virology* 200:524-534, 1994; Chazal, N., et al., *Virology* 68(1):111-122, 1994; Griffiths, J.C., et al., *J. Virol.* 67(6):3191-3198, 1993; Reicin, A.S., et al., *J. Virol.* 69(2):642-650, 1995).
- 15 Up to 50% of the coding sequences of p55Gag can be deleted without affecting the assembly to virus-like particles and expression efficiency (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Gamier, L., et al., *J Virol* 72(6):4667-4677, 1998; Zhang, Y., et al., *J Virol* 72(3):1782-1789, 1998; Wang, C., et al., *J Virol* 72(10): 7950-7959, 1998). In one embodiment of the present invention, immunogenicity of the high level expressing synthetic Gag expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted regions of Gag sequence.
- 20 Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this
- 30

approach, relative to using full-length sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency of the expression product.

When sequences are added to the amino terminal end of Gag, the polynucleotide can contain coding sequences at the 5' end that encode a signal for addition of a myristic moiety to the Gag-containing polypeptide (e.g., sequences that encode Met-Gly).

The ability of Gag-containing polypeptide constructs to form VLPs can be empirically determined following the teachings of the present specification.

The synthetic expression cassettes can also include control elements operably linked to the coding sequence, which allow for the expression of the gene *in vivo* in the subject species. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter, the mouse mammary tumor virus LTR promoter, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence.

Furthermore, plasmids can be constructed which include a chimeric antigen-coding gene sequences, encoding, e.g., multiple antigens/epitopes of interest, for example derived from more than one viral isolate.

Typically the antigen coding sequences precede or follow the synthetic coding sequence and the chimeric transcription unit will have a single open reading frame encoding both the antigen of interest and the synthetic coding sequences.

Alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like (Example 7).

In one embodiment of the present invention, a nucleic acid immunizing composition may comprise, for example, the following: a first expression vector comprising a Gag expression cassette, a second vector comprising an Env expression cassette, and a third expression vector comprising a Pol expression cassette, or one or more coding region of Pol (e.g., Prot, RT, RNase, Int), wherein further antigen coding sequences may be associated with the Pol expression, such antigens may be obtained, for example, from accessory genes (e.g., vpr, vpu, vif), regulatory genes (e.g., nef, tat, rev), or portions of the Pol sequences (e.g., Prot, RT, RNase, Int)). In another embodiment, a nucleic acid immunizing composition may comprise, for example, an expression cassette comprising any of the synthetic polynucleotide sequences of the present invention. In another embodiment, a nucleic acid immunizing composition may comprise, for example, an expression cassette comprising coding sequences for a number of HIV genes (or sequences derived from such genes) wherein the coding sequences are in-frame and under the control of a single promoter, for example, Gag-Env constructs, Tat-Rev-Nef constructs, P2Pol-tat-rev-nef constructs, etc. The synthetic coding sequences of the present invention may be combined in any number of combinations depending on the coding sequence products (i.e., HIV polypeptides) to which, for example, an immunological response is desired to be raised. In yet another embodiment, synthetic coding sequences for multiple HIV-derived polypeptides may be constructed into a polycistronic message under the control of a single promoter wherein IRES are placed adjacent the coding sequence for each encoded polypeptide.

Once complete, the constructs are used for nucleic acid immunization using standard gene delivery protocols. Methods for gene delivery are known in the art.

See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered

either directly to the vertebrate subject or, alternatively, delivered *ex vivo*, to cells derived from the subject and the cells reimplanted in the subject.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene
5 delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy*
10 (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus
15 minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

20 Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988)
25 8:3988-3996; Vincent et al., *Vaccines 90* (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

Another vector system useful for delivering the polynucleotides of the present invention is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued October 14, 1997).

Additional viral vectors which will find use for delivering the nucleic acid molecules encoding the antigens of interest include those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the particular synthetic HIV polypeptide coding sequence is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK⁻ recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545.

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as viral vectors for delivering the polynucleotides of the present invention (for

example, a synthetic Gag-polypeptide encoding expression cassette). For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T.W., et al., U.S. Patent No. 5,843,723, issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998. Preferred expression systems include, but are not limited to, eucaryotic layered vector initiation systems (e.g., US Patent No. 6,015,686, US Patent No. 5,814,482, US Patent No. 6,015,694, US Patent No. 5,789,245, EP 1029068A2, WO 9918226A2/A3, EP 00907746A2, WO 9738087A2).

10 A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest in a host cell. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters.

15 Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation

20 products. See, e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

 As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into

25 host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase which in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from

30 translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7

RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

Delivery of the expression cassettes of the present invention can also be accomplished using eucaryotic expression vectors comprising CMV-derived elements, such vectors include, but are not limited to, the following: pCMVKm2, pCMV-link pCMVPLEdhfr, and pCMV6a (all described above).

Synthetic expression cassettes of interest can also be delivered without a viral vector. For example, the synthetic expression cassette can be packaged in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta.* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et

al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in *METHODS OF IMMUNOLOGY* (1983), Vol. 101, pp. 512-527; Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77; Deamer and Bangham, *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348; Enoch and Strittmatter, *Proc. Natl. Acad. Sci. USA* (1979) 76:145; Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka and Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* (1978) 75:145; and Schaefer-Ridder et al., *Science* (1982) 215:166.

The DNA and/or protein antigen(s) can also be delivered in cochleate lipid compositions similar to those described by Papahadjopoulos et al., *Biochem. Biophys. Acta* (1975) 394:483-491. See, also, U.S. Patent Nos. 4,663,161 and 4,871,488.

The synthetic expression cassette of interest may also be encapsulated, adsorbed to, or associated with, particulate carriers. Such carriers present multiple copies of a selected antigen to the immune system and promote trapping and retention

of antigens in local lymph nodes. The particles can be phagocytosed by macrophages and can enhance antigen presentation through cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Suitable microparticles may also be manufactured in the presence of charged detergents, such as anionic or cationic detergents, to yield microparticles with a surface having a net negative or a net positive charge. For example, microparticles manufactured with anionic detergents, such as hexadecyltrimethylammonium bromide (CTAB), i.e. CTAB-PLG microparticles, adsorb negatively charged macromolecules, such as DNA. (see, e.g., Int'l Application Number PCT/US99/17308).

Furthermore, other particulate systems and polymers can be used for the *in vivo* or *ex vivo* delivery of the gene of interest. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued November 3, 1998) may also be used for delivery of a construct of the present invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering synthetic expression cassettes of the present invention. The particles are coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-

less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

Recombinant vectors carrying a synthetic expression cassette of the present invention are formulated into compositions for delivery to the vertebrate subject.

5 These compositions may either be prophylactic (to prevent infection) or therapeutic (to treat disease after infection). The compositions will comprise a "therapeutically effective amount" of the gene of interest such that an amount of the antigen can be produced *in vivo* so that an immune response is generated in the individual to which it is administered. The exact amount necessary will vary depending on the subject being
10 treated; the age and general condition of the subject to be treated; the capacity of the subject's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular antigen selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. Thus, a "therapeutically effective amount" will
15 fall in a relatively broad range that can be determined through routine trials.

The compositions will generally include one or more "pharmaceutically acceptable excipients or vehicles" such as water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such
20 vehicles. Certain facilitators of nucleic acid uptake and/or expression can also be included in the compositions or coadministered, such as, but not limited to, bupivacaine, cardiotoxin and sucrose.

Once formulated, the compositions of the invention can be administered directly to the subject (e.g., as described above) or, alternatively, delivered *ex vivo*, to
25 cells derived from the subject, using methods such as those described above. For example, methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and can include, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, lipofectamine and LT-1 mediated transfection, protoplast fusion, electroporation, encapsulation of the
30 polynucleotide(s) (with or without the corresponding antigen) in liposomes, and direct microinjection of the DNA into nuclei.

Direct delivery of synthetic expression cassette compositions *in vivo* will generally be accomplished with or without viral vectors, as described above, by injection using either a conventional syringe or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). The constructs
5 can be injected either subcutaneously, epidermally, intradermally, intramucosally such as nasally, rectally and vaginally, intraperitoneally, intravenously, orally or intramuscularly. Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient presence of DNA in the recipient. Other modes of
10 administration include oral and pulmonary administration, suppositories, needle-less injection, transcutaneous and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. Administration of nucleic acids may also be combined with administration of peptides or other substances.

Exemplary immunogenicity studies are presented in Examples 4, 5, 6, 9, 10,
15 11, and 12.

2.4.2 EX VIVO DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

In one embodiment, T cells, and related cell types (including but not limited to
20 antigen presenting cells, such as, macrophage, monocytes, lymphoid cells, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), can be used for *ex vivo* delivery of the synthetic expression cassettes of the present invention. T cells can be isolated from peripheral blood lymphocytes (PBLs) by a variety of procedures known to those skilled in the art. For example, T cell populations can be “enriched” from a
25 population of PBLs through the removal of accessory and B cells. In particular, T cell enrichment can be accomplished by the elimination of non-T cells using anti-MHC class II monoclonal antibodies. Similarly, other antibodies can be used to deplete specific populations of non-T cells. For example, anti-Ig antibody molecules can be used to deplete B cells and anti-MacI antibody molecules can be used to deplete
30 macrophages.

T cells can be further fractionated into a number of different subpopulations by techniques known to those skilled in the art. Two major subpopulations can be isolated based on their differential expression of the cell surface markers CD4 and CD8. For example, following the enrichment of T cells as described above, CD4⁺ cells
5 can be enriched using antibodies specific for CD4 (see Coligan et al., *supra*). The antibodies may be coupled to a solid support such as magnetic beads. Conversely, CD8⁺ cells can be enriched through the use of antibodies specific for CD4 (to remove CD4⁺ cells), or can be isolated by the use of CD8 antibodies coupled to a solid support. CD4 lymphocytes from HIV-1 infected patients can be expanded *ex vivo*,
10 before or after transduction as described by Wilson et. al. (1995) *J. Infect. Dis.* 172:88.

Following purification of T cells, a variety of methods of genetic modification known to those skilled in the art can be performed using non-viral or viral-based gene transfer vectors constructed as described herein. For example, one such approach
15 involves transduction of the purified T cell population with vector-containing supernatant of cultures derived from vector producing cells. A second approach involves co-cultivation of an irradiated monolayer of vector-producing cells with the purified T cells. A third approach involves a similar co-cultivation approach; however, the purified T cells are pre-stimulated with various cytokines and cultured 48 hours
20 prior to the co-cultivation with the irradiated vector producing cells. Pre-stimulation prior to such transduction increases effective gene transfer (Nolta et al. (1992) *Exp. Hematol.* 20:1065). Stimulation of these cultures to proliferate also provides increased cell populations for re-infusion into the patient. Subsequent to co-cultivation, T cells are collected from the vector producing cell monolayer, expanded,
25 and frozen in liquid nitrogen.

Gene transfer vectors, containing one or more synthetic expression cassette of the present invention (associated with appropriate control elements for delivery to the isolated T cells) can be assembled using known methods and following the guidance of the present specification.

30 Selectable markers can also be used in the construction of gene transfer vectors. For example, a marker can be used which imparts to a mammalian cell

transduced with the gene transfer vector resistance to a cytotoxic agent. The cytotoxic agent can be, but is not limited to, neomycin, aminoglycoside, tetracycline, chloramphenicol, sulfonamide, actinomycin, netropsin, distamycin A, anthracycline, or pyrazinamide. For example, neomycin phosphotransferase II imparts resistance to the
5 neomycin analogue geneticin (G418).

The T cells can also be maintained in a medium containing at least one type of growth factor prior to being selected. A variety of growth factors are known in the art which sustain the growth of a particular cell type. Examples of such growth factors are cytokine mitogens such as rIL-2, IL-10, IL-12, and IL-15, which promote growth
10 and activation of lymphocytes. Certain types of cells are stimulated by other growth factors such as hormones, including human chorionic gonadotropin (hCG) and human growth hormone. The selection of an appropriate growth factor for a particular cell population is readily accomplished by one of skill in the art.

For example, white blood cells such as differentiated progenitor and stem cells
15 are stimulated by a variety of growth factors. More particularly, IL-3, IL-4, IL-5, IL-6, IL-9, GM-CSF, M-CSF, and G-CSF, produced by activated T_H and activated macrophages, stimulate myeloid stem cells, which then differentiate into pluripotent stem cells, granulocyte-monocyte progenitors, eosinophil progenitors, basophil progenitors, megakaryocytes, and erythroid progenitors. Differentiation is modulated
20 by growth factors such as GM-CSF, IL-3, IL-6, IL-11, and EPO.

Pluripotent stem cells then differentiate into lymphoid stem cells, bone marrow stromal cells, T cell progenitors, B cell progenitors, thymocytes, T_H Cells, T_C cells, and B cells. This differentiation is modulated by growth factors such as IL-3, IL-4, IL-6, IL-7, GM-CSF, M-CSF, G-CSF, IL-2, and IL-5.

25 Granulocyte-monocyte progenitors differentiate to monocytes, macrophages, and neutrophils. Such differentiation is modulated by the growth factors GM-CSF, M-CSF, and IL-8. Eosinophil progenitors differentiate into eosinophils. This process is modulated by GM-CSF and IL-5.

The differentiation of basophil progenitors into mast cells and basophils is
30 modulated by GM-CSF, IL-4, and IL-9. Megakaryocytes produce platelets in

response to GM-CSF, EPO, and IL-6. Erythroid progenitor cells differentiate into red blood cells in response to EPO.

Thus, during activation by the CD3-binding agent, T cells can also be contacted with a mitogen, for example a cytokine such as IL-2. In particularly preferred embodiments, the IL-2 is added to the population of T cells at a concentration of about 50 to 100 $\mu\text{g/ml}$. Activation with the CD3-binding agent can be carried out for 2 to 4 days.

Once suitably activated, the T cells are genetically modified by contacting the same with a suitable gene transfer vector under conditions that allow for transfection of the vectors into the T cells. Genetic modification is carried out when the cell density of the T cell population is between about 0.1×10^6 and 5×10^6 , preferably between about 0.5×10^6 and 2×10^6 . A number of suitable viral and nonviral-based gene transfer vectors have been described for use herein.

After transduction, transduced cells are selected away from non-transduced cells using known techniques. For example, if the gene transfer vector used in the transduction includes a selectable marker which confers resistance to a cytotoxic agent, the cells can be contacted with the appropriate cytotoxic agent, whereby non-transduced cells can be negatively selected away from the transduced cells. If the selectable marker is a cell surface marker, the cells can be contacted with a binding agent specific for the particular cell surface marker, whereby the transduced cells can be positively selected away from the population. The selection step can also entail fluorescence-activated cell sorting (FACS) techniques, such as where FACS is used to select cells from the population containing a particular surface marker, or the selection step can entail the use of magnetically responsive particles as retrievable supports for target cell capture and/or background removal.

More particularly, positive selection of the transduced cells can be performed using a FACS cell sorter (e.g. a FACSVantage™ Cell Sorter, Becton Dickinson Immunocytometry Systems, San Jose, CA) to sort and collect transduced cells expressing a selectable cell surface marker. Following transduction, the cells are stained with fluorescent-labeled antibody molecules directed against the particular cell surface marker. The amount of bound antibody on each cell can be measured by

passing droplets containing the cells through the cell sorter. By imparting an electromagnetic charge to droplets containing the stained cells, the transduced cells can be separated from other cells. The positively selected cells are then harvested in sterile collection vessels. These cell sorting procedures are described in detail, for example, in the FACSVantage™ Training Manual, with particular reference to sections 3-11 to 3-28 and 10-1 to 10-17.

Positive selection of the transduced cells can also be performed using magnetic separation of cells based on expression of a particular cell surface marker. In such separation techniques, cells to be positively selected are first contacted with specific binding agent (e.g., an antibody or reagent that interacts specifically with the cell surface marker). The cells are then contacted with retrievable particles (e.g., magnetically responsive particles) which are coupled with a reagent that binds the specific binding agent (that has bound to the positive cells). The cell-binding agent-particle complex can then be physically separated from non-labeled cells, for example using a magnetic field. When using magnetically responsive particles, the labeled cells can be retained in a container using a magnetic field while the negative cells are removed. These and similar separation procedures are known to those of ordinary skill in the art.

Expression of the vector in the selected transduced cells can be assessed by a number of assays known to those skilled in the art. For example, Western blot or Northern analysis can be employed depending on the nature of the inserted nucleotide sequence of interest. Once expression has been established and the transformed T cells have been tested for the presence of the selected synthetic expression cassette, they are ready for infusion into a patient via the peripheral blood stream.

The invention includes a kit for genetic modification of an *ex vivo* population of primary mammalian cells. The kit typically contains a gene transfer vector coding for at least one selectable marker and at least one synthetic expression cassette contained in one or more containers, ancillary reagents or hardware, and instructions for use of the kit.

30

2.4.3 FURTHER DELIVERY REGIMES

Any of the polynucleotides (*e.g.*, expression cassettes) or polypeptides described herein (delivered by any of the methods described above) can also be used in combination with other DNA delivery systems and/or protein delivery systems. Non-limiting examples include co-administration of these molecules, for example, in prime-boost methods where one or more molecules are delivered in a “priming” step and, subsequently, one or more molecules are delivered in a “boosting” step. In certain embodiments, the delivery of one or more nucleic acid-containing compositions and is followed by delivery of one or more nucleic acid-containing compositions and/or one or more polypeptide-containing compositions (*e.g.*, polypeptides comprising HIV antigens). In other embodiments, multiple nucleic acid “primes” (of the same or different nucleic acid molecules) can be followed by multiple polypeptide “boosts” (of the same or different polypeptides). Other examples include multiple nucleic acid administrations and multiple polypeptide administrations.

In any method involving co-administration, the various compositions can be delivered in any order. Thus, in embodiments including delivery of multiple different compositions or molecules, the nucleic acids need not be all delivered before the polypeptides. For example, the priming step may include delivery of one or more polypeptides and the boosting comprises delivery of one or more nucleic acids and/or one more polypeptides. Multiple polypeptide administrations can be followed by multiple nucleic acid administrations or polypeptide and nucleic acid administrations can be performed in any order. In any of the embodiments described herein, the nucleic acid molecules can encode all, some or none of the polypeptides. Thus, one or more of the nucleic acid molecules (*e.g.*, expression cassettes) described herein and/or one or more of the polypeptides described herein can be co-administered in any order and via any administration routes. Therefore, any combination of polynucleotides and/or polypeptides described herein can be used to generate elicit an immune reaction.

30

3.0 IMPROVED HIV-1 GAG AND POL EXPRESSION CASSETTES

While not desiring to be bound by any particular model, theory, or hypothesis, the following information is presented to provide a more complete understanding of the present invention.

5 The world health organization (WHO) estimated the number of people worldwide that are infected with HIV-1 to exceed 36.1 million. The development of a safe and effective HIV vaccine is therefore essential at this time. Recent studies have demonstrated the importance of CTL in controlling the HIV-1 replication in infected patients. Furthermore, CTL reactivity with multiple HIV antigens will be necessary for
10 the effective control of virus replication. Experiments performed in support of the present invention suggest that the inclusion of HIV-1 Gag and Pol, beside Env for the induction of neutralizing antibodies, into the vaccine is useful.

 To increase the potency of HIV-1 vaccine candidates, codon modified Gag and Pol expression cassettes were designed, either for Gag alone or Gag plus Pol. To
15 evaluate possible differences in expression and potency, the expression of these constructs was analyzed and immunogenicity studies carried out in mice.

 Several expression cassettes encoding Gag and Pol were designed, including, but not limited to, the following: GagProtease, GagPol Δ integrase with frameshift (gagFSpol), and GagPol Δ integrase in-frame (gagpol). Versions of GagPol Δ integrase
20 in-frame were also designed with attenuated (Att) or non-functional Protease (Ina). The nucleic acid sequences were codon modified to correspond to the codon usage of highly expressed human genes. Mice were immunized with titrated DNA doses and humoral and cellular immune responses evaluated by ELISA and intracellular cytokine staining (Example 10).

25 The immune responses in mice has been seen to be correlated with relative levels of expression *in vitro*. Vaccine studies in rhesus monkeys will further address immune responses and expression levels *in vivo*.

4.0 ENHANCED VACCINE TECHNOLOGIES FOR THE INDUCTION OF POTENT NEUTRALIZING ANTIBODIES AND CELLULAR IMMUNE RESPONSES AGAINST HIV.

While not desiring to be bound by any particular model, theory, or hypothesis,
5 the following information is presented to provide a more complete understanding of
the present invention.

Protection against HIV infection will likely require potent and broadly reactive
pre-existing neutralizing antibodies in vaccinated individuals exposed to a virus
challenge. Although cellular immune responses are desirable to control viremia in
10 those who get infected, protection against infection has not been demonstrated for
vaccine approaches that rely exclusively on the induction of these responses. For this
reason, experiments performed in support of the present invention use prime-boost
approaches that employ novel V-deleted envelope antigens from primary HIV isolates
(e.g., R5 subtype B (HIV-1_{SF162}) and subtype C (HIV-1_{TV1}) strains). These antigens
15 were delivered by enhanced DNA [polyactide co-glycolide (PLG) microparticle
formulations or electroporation] or alphavirus replicon particle-based vaccine
approaches, followed by booster immunizations with Env proteins in MF59 adjuvant.
Efficient in vivo expression of plasmid encoded genes by electrical permeabilization
has been described (see, e.g., Zucchelli et al. (2000) *J. Virol.* 74:11598-11607; Banga
20 et al. (1998) *Trends Biotechnol.* 10:408-412; Heller et al. (1996) *Febs Lett.* 389:225-
228; Mathiesen et al. (1999) *Gene Ther.* 4:508-514; Mir et al. (1999) *Proc. Nat'l Acad
Sci. USA* 8:4262-4267; Nishi et al. (1996) *Cancer Res.* 5:1050-1055). Both native
and V-deleted monomeric (gp120) and oligomeric (o-gp140) forms of protein from the
SF162 strain were tested as boosters. All protein preparations were highly purified
25 and extensively characterized by biophysical and immunochemical methodologies.
Results from rabbit and primate immunogenicity studies indicated that, whereas
neutralizing antibody responses could be consistently induced against the parental non-
V2-deleted SF162 virus, the induction of responses against heterologous HIV strains
improved with deletion of the V2 loop of the immunogens. Moreover, using these
30 prime-boost vaccine regimens, potent HIV antigen-specific CD4 + and CD8+ T-cell
responses were also demonstrated.

Based on these findings, V2-deleted envelope DNA and protein vaccines were chosen for advancement toward clinical evaluation. Similar approaches for immunization may be employed using, for example, nucleic acid immunization employing the synthetic HIV polynucleotides of the present invention coupled with
5 corresponding or heterologous HIV-derived polypeptide boosts.

One embodiment of this aspect of the present invention may be described generally as follows. Antigens are selected for the vaccine composition(s). Env polypeptides are typically employed in a first antigenic composition used to induce an immune response. Further, Gag polypeptides are typically employed in a second
10 antigenic composition used to induce an immune response. The second antigenic composition may include further HIV-derived polypeptide sequences, including, but not limited to, Pol, Tat, Rev, Nef, Vif, Vpr, and/or Vpu sequences. A DNA prime vaccination is typically performed with the first and second antigenic compositions. Further DNA vaccinations with one or more of the antigenic compositions may also be
15 included at selected time intervals. The prime is typically followed by at least one boost. The boost may, for example, include adjuvanted HIV-derived polypeptides (e.g., corresponding to those used for the DNA vaccinations), coding sequences for HIV-derived polypeptides (e.g., corresponding to those used for the DNA vaccinations) encoded by a viral vector, further DNA vaccinations, and/or
20 combinations of the foregoing. In one embodiment, a DNA prime is administered with a first antigenic composition (e.g., a DNA construct encoding an Envelope polypeptide) and second antigenic composition (e.g., a DNA construct encoding a Gag polypeptide, a Pol polypeptide, a Tat polypeptide, a Nef polypeptide, and a Rev polypeptide). The DNA construct for use in the prime may, for example, comprise a
25 CMV promoter operably linked to the polynucleotide encoding the polypeptide sequence. The DNA prime is followed by a boost, for example, an adjuvanted Envelope polypeptide boost and a viral vector boost (where the viral vector encodes, e.g., a Gag polypeptide, a Pol polypeptide, a Tat polypeptide, a Nef polypeptide, and a Rev polypeptide). Alternately (or in addition), the boost may be an adjuvanted Gag
30 polypeptide, Pol polypeptide, Tat polypeptide, Nef polypeptide, and Rev polypeptide boost and a viral vector boost (where the viral vector encodes, e.g., an Envelope

polypeptide). The boost may include all polypeptide antigens which were encoded in the DNA prime; however, this is not required. Further, different polypeptide antigens may be used in the boost relative to the initial vaccination and visa versa. Further, the initial vaccination may be a viral vector rather than a DNA construct.

5 Some factors that may be considered in HIV envelope vaccine design are as follows. Envelope-based vaccines have demonstrated protection against infection in non-human primate models. Passive antibody studies have demonstrated protection against HIV infection in the presence of neutralizing antibodies against the virus challenge stock. Vaccines that exclude Env generally confer less protective efficacy.

10 Experiments performed in support of the present invention have demonstrated that monomeric gp120 protein-derived from the SF2 lab strain provided neutralization of HIV-1 lab strains and protection against virus challenges in primate models. Primary gp120 protein derived from Thai E field strains provided cross-subtype neutralization of lab strains. Primary sub-type B oligomeric o-gp140 protein provided partial

15 neutralization of subtype B primary (field) isolates. Primary sub-type B o-gp140ΔV2 DNA prime plus protein boost provided potent neutralization of diverse subtype B primary isolates and protection against virus challenge in primate models. Primary sub-type C o-gp140 and o-gp140ΔV2 likely provide similar results to those just described for sub-type B.

20 Vaccine strategies for induction of potent, broadly reactive, neutralizing antibodies may be assisted by construction of Envelope polypeptide structures that expose conserved neutralizing epitopes, for example, variable-region deletions and de-glycosylations, envelope protein-receptor complexes, rational design based on crystal structure (e.g., β-sheet deletions), and gp41-fusion domain based immunogens.

25 Stable CHO cell lines for envelope protein production have been developed using optimized envelope polypeptide coding sequences, including, but not limited to, the following: gp120, o-gp140, gp120ΔV2, o-gp140ΔV2, gp120ΔV1V2, o-gp140ΔV1V2.

 In addition, following prime-boost regimes (such as those described above)

30 appear to be beneficial to help reduce viral load in infected subjects, as well as possibly slow or prevent progression of HIV-related disease (relative to untreated subjects).

Exemplary antigenic compositions and immunogenicity studies are presented in Examples 9, 10, 11, and 12.

EXPERIMENTAL

5 Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of
10 course, be allowed for.

Example 1

Generation of Synthetic Expression Cassettes

A. Generating Synthetic Polynucleotides

15 The polynucleotide sequences of the present invention were manipulated to maximize expression of their gene products. The order of the following steps may vary.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed
20 human genes. The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a high AU content in the RNA and in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The wild-type sequences were modified
25 to be comparable to codon usage found in highly expressed human genes.

Second, for some genes non-functional variants were created. In the following table (Table B) mutations affecting the activity of several HIV genes are disclosed.

Table B

Gene	"Region"	Exemplary Mutations
Pol	prot	<p>Att = Reduced activity by attenuation of Protease (Thr26Ser) (e.g., Konvalinka et al., 1995, J Virol 69: 7180-86)</p> <p>Ina = Mutated Protease, nonfunctional enzyme (Asp25Ala)(e.g., Konvalinka et al., 1995, J Virol 69: 7180-86)</p>
	RT	<p>YM = Deletion of catalytic center (YMDD_AP; SEQ ID NO:7) (e.g., Biochemistry, 1995, 34, 5351, Patel et. al.)</p> <p>WM = Deletion of primer grip region (WMGY_PI; SEQ ID NO:8)) (e.g., J Biol Chem, 272, 17, 11157, Palaniappan, et. al., 1997)</p>
	RNase	no direct mutations, RnaseH is affected by "WM" mutation in RT
	Integrase	<p>1) Mutation of HHCC domain, Cys40Ala (e.g., Wiskerchen et. al., 1995, J Virol, 69: 376).</p> <p>2.) Inactivation catalytic center, Asp64Ala, Asp116Ala, Glu152Ala (e.g., Wiskerchen et. al., 1995, J Virol, 69: 376).</p> <p>3) Inactivation of minimal DNA binding domain (MDBD), deletion of Trp235(e.g., Ishikawa et. al., 1999, J Virol, 73: 4475).</p> <p>Constructs int.opt.mut.SF2 and int.opt.mut_C (South Africa TV1) both contain all these mutations (1, 2, and 3)</p>
Env		<p>Mutations in cleavage site (e.g., mut1-4, 7)</p> <p>Mutations in glycosylation site (e.g., GM mutants, for example, change Q residue in V1 and/or V2 to N residue; may also be designated by residue altered in sequence)</p>
5 Tat		<p>Mutants of Tat in transactivation domain (e.g., Caputo et al., 1996, Gene Ther. 3:235)</p> <p>cys22 mutant (Cys22Gly) = TatC22</p> <p>cys37 mutant (Cys37Ser) = TatC37</p> <p>cys22/37 double mutant = TatC22/37</p>

Gene	"Region"	Exemplary Mutations
Rev		Mutations in Rev domains (e.g., Thomas et al., 1998, J Virol. 72:2935-44) Mutation in RNA binding-nuclear localization ArgArg38,39AspLeu = M5 Mutation in activation domain LeuGlu78,79AspLeu = M10
Nef		Mutations of myristoylation signal and in oligomerization domain: 1. Single point mutation myristoylation signal: Gly-to-Ala = -Myr 2. Deletion of N-terminal first 18 (sub-type B, e.g., SF162) or 19 (sub-type C, e.g., South Africa clones) amino acids: -Myr18 or -Myr19 (respectively) (e.g., Peng and Robert-Guroff, 2001, Immunol Letters 78: 195-200) Single point mutation oligomerization: (e.g., Liu et al., 2000, J Virol 74: 5310-19) Asp125Gly (sub B SF162) or Asp124Gly (sub C South Africa clones) Mutations affecting (1) infectivity (replication) of HIV-virions and/or (2) CD4 down regulation. (e.g., Lundquist et al. (2002) J Virol. 76(9):4625-33)
Vif		Mutations of Vif: e.g., Simon et al., 1999, J Virol 73:2675-81
Vpr		Mutations of Vpr: e.g., Singh et al., 2000, J Virol 74: 10650-57
Vpu		Mutations of Vpu: e.g., Tiganos et al., 1998, Virology 251: 96-107

5

Constructs comprising some of these mutations are described herein. Vif, vpr and vpu synthetic constructs are described. Reducing or eliminating the function of the associated gene products can be accomplished employing the teachings set forth in the above table, in view of the teachings of the present specification.

10

In one embodiment of the invention, the full length coding region of the Gag-polymerase sequence is included with the synthetic Gag sequences in order to increase

the number of epitopes for virus-like particles expressed by the synthetic, optimized Gag expression cassette. Because synthetic HIV-1 Gag-polymerase expresses the potentially deleterious functional enzymes reverse transcriptase (RT) and integrase (INT) (in addition to the structural proteins and protease), it is important to inactivate

5 RT and INT functions. Several in-frame deletions in the RT and INT reading frame can be made to achieve catalytic nonfunctional enzymes with respect to their RT and INT activity. {Jay. A. Levy (Editor) (1995) *The Retroviridae*, Plenum Press, New York. ISBN 0-306-45033X. Pages 215-20; Grimson, B. and Laurence, J. (1995), *Journal Of Acquired Immune Deficiency Syndromes and Human Retrovirology*

10 9(1):58-68; Wakefield, J. K., et al., (1992) *Journal Of Virology* 66(11):6806-6812; Esnouf, R., et al., (1995) *Nature Structural Biology* 2(4):303-308; Maignan, S., et al., (1998) *Journal Of Molecular Biology* 282(2):359-368; Katz, R. A. and Skalka, A. M. (1994) *Annual Review Of Biochemistry* 73 (1994); Jacobo-Molina, A., et al., (1993) *Proceedings Of the National Academy Of Sciences Of the United States Of America*

15 90(13):6320-6324; Hickman, A. B., et al., (1994) *Journal Of Biological Chemistry* 269(46):29279-29287; Goldgur, Y., et al., (1998) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 95(16):9150-9154; Goette, M., et al., (1998) *Journal Of Biological Chemistry* 273(17):10139-10146; Gorton, J. L., et al., (1998) *Journal of Virology* 72(6):5046-5055; Engelman, A., et al., (1997)

20 *Journal Of Virology* 71(5):3507-3514; Dyda, F., et al., *Science* 266(5193):1981-1986; Davies, J. F., et al., (1991) *Science* 252(5002):88-95; Bujacz, G., et al., (1996) *Febs Letters* 398(2-3):175-178; Beard, W. A., et al., (1996) *Journal Of Biological Chemistry* 271(21):12213-12220; Kohlstaedt, L. A., et al., (1992) *Science* 256(5065):1783-1790; Krug, M. S. and Berger, S. L. (1991) *Biochemistry*

25 30(44):10614-10623; Mazumder, A., et al., (1996) *Molecular Pharmacology* 49(4):621-628; Palaniappan, C., et al., (1997) *Journal Of Biological Chemistry* 272(17):11157-11164; Rodgers, D. W., et al., (1995) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 92(4):1222-1226; Sheng, N. and Dennis, D. (1993) *Biochemistry* 32(18):4938-4942; Spence, R. A., et al., (1995)

30 *Science* 267(5200):988-993.}

Furthermore selected B- and/or T-cell epitopes can be added to the Gag-polymerase constructs within the deletions of the RT- and INT-coding sequence to replace and augment any epitopes deleted by the functional modifications of RT and INT. Alternately, selected B- and T-cell epitopes (including CTL epitopes) from RT and INT can be included in a minimal VLP formed by expression of the synthetic Gag or synthetic GagProt cassette, described above. (For descriptions of known HIV B- and T-cell epitopes see, HIV Molecular Immunology Database CTL Search Interface; Los Alamos Sequence Compendia, 1987-1997; Internet address: <http://hiv-web.lanl.gov/immunology/index.html>.)

In another aspect, the present invention comprises *Env* coding sequences that include, but are not limited to, polynucleotide sequences encoding the following HIV-encoded polypeptides: gp160, gp140, and gp120 (see, e.g., U.S. Patent No. 5,792,459 for a description of the HIV-1_{SF2} ("SF2") *Env* polypeptide). The relationships between these polypeptides is shown schematically in Figure 3 (in the figure: the polypeptides are indicated as lines, the amino and carboxy termini are indicated on the gp160 line; the open circle represents the oligomerization domain; the open square represents a transmembrane spanning domain (TM); and "c" represents the location of a cleavage site, in gp140.mut the "X" indicates that the cleavage site has been mutated such that it no longer functions as a cleavage site). The polypeptide gp160 includes the coding sequences for gp120 and gp41. The polypeptide gp41 is comprised of several domains including an oligomerization domain (OD) and a transmembrane spanning domain (TM). In the native envelope, the oligomerization domain is required for the non-covalent association of three gp41 polypeptides to form a trimeric structure: through non-covalent interactions with the gp41 trimer (and itself), the gp120 polypeptides are also organized in a trimeric structure. A cleavage site (or cleavage sites) exists approximately between the polypeptide sequences for gp120 and the polypeptide sequences corresponding to gp41. This cleavage site(s) can be mutated to prevent cleavage at the site. The resulting gp140 polypeptide corresponds to a truncated form of gp160 where the transmembrane spanning domain of gp41 has been deleted. This gp140 polypeptide can exist in both monomeric and oligomeric (*i.e.* trimeric) forms by virtue of the presence of the oligomerization domain in the gp41 moiety. In the

situation where the cleavage site has been mutated to prevent cleavage and the transmembrane portion of gp41 has been deleted the resulting polypeptide product is designated "mutated" gp140 (e.g., gp140.mut). As will be apparent to those in the field, the cleavage site can be mutated in a variety of ways. (See, also, WO 00/39302).

5 Wild-type HIV coding sequences (e.g., Gag, Env, Pol, tat, rev, nef, vpr, vpu, vif, etc.) can be selected from any known HIV isolate and these sequences manipulated to maximize expression of their gene products following the teachings of the present invention. The wild-type coding region maybe modified in one or more of the following ways. In one embodiment, sequences encoding hypervariable regions of
10 Env, particularly V1 and/or V2 were deleted. In other embodiments, mutations were introduced into sequences, for example, encoding the cleavage site in Env to abrogate the enzymatic cleavage of oligomeric gp140 into gp120 monomers. (See, e.g., Earl et al. (1990) *PNAS USA* 87:648-652; Earl et al. (1991) *J. Virol.* 65:31-41). In yet other embodiments, hypervariable region(s) were
15 deleted, N-glycosylation sites were removed and/or cleavage sites mutated. As discussed above, different mutations may be introduced into the coding sequences of different genes (see, e.g., Table B). For example, *Tat* coding sequences were modified according to the teachings of the present specification, for example to affect the transactivation domain of the gene product (e.g., replacing a cystein residue at position
20 22 with a glycine, Caputo et al. (1996) *Gene Therapy* 3:235).

To create the synthetic coding sequences of the present invention the gene cassettes are designed to comprise the entire coding sequence of interest. Synthetic gene cassettes are constructed by oligonucleotide synthesis and PCR amplification to generate gene fragments. Primers are chosen to provide convenient restriction sites
25 for subcloning. The resulting fragments are then ligated to create the entire desired sequence which is then cloned into an appropriate vector. The final synthetic sequences are (i) screened by restriction endonuclease digestion and analysis, (ii) subjected to DNA sequencing in order to confirm that the desired sequence has been obtained and (iii) the identity and integrity of the expressed protein confirmed by SDS-
30 PAGE and Western blotting. The synthetic coding sequences are assembled at Chiron

Corp. (Emeryville, CA) or by the Midland Certified Reagent Company (Midland, Texas).

Percent identity to the synthetic sequences of the present invention can be determined, for example, using the Smith-Waterman search algorithm (Time Logic, Incline Village, NV), with the following exemplary parameters: weight matrix = nuc4x4hb; gap opening penalty = 20, gap extension penalty = 5, reporting threshold = 1; alignment threshold = 20.

Various forms of the different embodiments of the present invention (*e.g.*, constructs) may be combined.

Exemplary embodiments of the synthetic polynucleotides of the present invention include, but are not limited to, the sequences presented in Table C.

Table C

Type C Synthetic, Codon Optimized Polynucleotides

	Name	Figure Number	Description (encoding)
15	GagComplPolmut_C (SEQ ID NO:9)	6	Gag complete, Pol, RT mutated; all in-frame
	GagComplPolmutAtt_C (SEQ ID NO:10)	7	Gag complete, Pol, RT mutated, protease attenuated; all in-frame
20	GagComplPolmutIna_C (SEQ ID NO:11)	8	Gag complete, Pol, RT mutated, protease non-functional; all in-frame
	GagComplPolmutInaTatRevNef_C (SEQ ID NO:12)	9	Gag complete, Pol, RT mutated, protease non-functional, tat mutated, rev mutated, nef mutated; all in-frame
	GagPolmut_C (SEQ ID NO:13)	10	Gag, Pol, RT mutated; all in-frame
25	GagPolmutAtt_C (SEQ ID NO:14)	11	Gag, Pol, RT mutated, protease attenuated; all in-frame
	GagPolmutIna_C (SEQ ID NO:15)	12	Gag, Pol, RT mutated, protease non-functional; all in-frame

	Name	Figure Number	Description (encoding)
	GagProtInaRTmut_C (SEQ ID NO:16)	13	Gag, protease non-functional, RT mutated; all in-frame
	GagProtInaRTmutTatRevNef_C (SEQ ID NO:17)	14	Gag, protease non-functional, RT mutated, tat mutated, rev mutated, nef mutated; all in-frame
5	GagRTmut_C (SEQ ID NO:18)	15	Gag, RT mutated; all in-frame
	GagRTmutTatRevNef_C (SEQ ID NO:19)	16	Gag, RT mutated, tat mutated, rev mutated, nef mutated; all in-frame
10	GagTatRevNef_C (SEQ ID NO:20)	17	Gag, tat mutated, rev mutated, nef mutated; all in-frame
	gp120mod.TV1.del118-210 (SEQ ID NO:21)	18	gp120 derived from TV1.c8.2, deleted V1/V2 loops and stem
	gp120mod.TV1.delV1V2 (SEQ ID NO:22)	19	gp120 derived from TV1.c8.2, deleted V1/V2 loops
15	gp120mod.TV1.delV2 (SEQ ID NO:23)	20	gp120 derived from TV1.c8.2, deleted V2 loop
	gp140mod.TV1.del118-210 (SEQ ID NO:24)	21	gp140 derived from TV1.c8.2, deleted V1/V2 loops and stem
20	gp140mod.TV1.delV1V2 (SEQ ID NO:25)	22	gp140 derived from TV1.c8.2, deleted V1/V2 loops
	gp140mod.TV1.delV2 (SEQ ID NO:26)	23	gp140 derived from TV1.c8.2, deleted V2 loop
	gp140mod.TV1.mut7 (SEQ ID NO:27)	24	gp140 derived from TV1.c8.2, mutated protease cleavage site
25	gp140mod.TV1.tpa2 (SEQ ID NO:28)	25	gp140 derived from TV1.c8.2, tpa2 leader sequence
	gp140TMmod.TV1 (SEQ ID NO:29)	26	gp140 derived from TV1.c8.2, containing the transmembrane region
30	gp160mod.TV1.del118-210 (SEQ ID NO:30)	27	gp160 derived from TV1.c8.2, deleted V1/V2 loops and stem

	Name	Figure Number	Description (encoding)
	gp160mod.TV1.delV1V2 (SEQ ID NO:31)	28	gp160 derived from TV1.c8.2, deleted V1/V2 loops
	gp160mod.TV1.delV2 (SEQ ID NO:32)	29	gp160 derived from TV1.c8.2, deleted V2 loop
5	gp160mod.TV1.dV1 (SEQ ID NO:33)	30	gp160 derived from TV1.c8.2, deleted V1 loop
	gp160mod.TV1.dV1- gagmod.BW965 (SEQ ID NO:34)	31	gp160 derived from TV1.c8.2, deleted V1 loop, Gag derived from BW965; all in-frame
10	gp160mod.TV1.dV1V2- gagmod.BW965 (SEQ ID NO:35)	32	gp160 derived from TV1.c8.2, deleted V1/V2 loops, Gag derived from BW965; all in- frame
	gp160mod.TV1.dV2- gagmod.BW965 (SEQ ID NO:36)	33	gp160 derived from TV1.c8.2, deleted V2 loop, Gag derived from BW965; all in-frame
15	gp160mod.TV1.tpa2 (SEQ ID NO:37)	34	gp160 derived from TV1.c8.2, tpa2 leader; all in-frame
	gp160mod.TV1-gagmod.BW965 (SEQ ID NO:38)	35	gp160 derived from TV1.c8.2, Gag derived from BW965; all in-frame
20	int.opt.mut_C (SEQ ID NO:39)	36	integrase mutated
	int.opt_C (SEQ ID NO:40)	37	integrase
	nef.D106G.-myr19.opt_C (SEQ ID NO:41)	38	nef mutated
25	p15RnaseH.opt_C (SEQ ID NO:42)	39	p15 RNase H; all in-frame
	p2Pol.opt.YMWM_C (SEQ ID NO:43)	40	p2 Pol, RT mutated YM WM; all in-frame
30	p2Polopt.YM_C (SEQ ID NO:44)	41	p2 pol, RT mutated YM; all in- frame
	p2Polopt_C (SEQ ID NO:45)	42	p2 Pol; all in-frame

	Name	Figure Number	Description (encoding)
	p2PolTatRevNef opt C (SEQ ID NO:46)	43	p2 Pol, RT mutated, protease non-functional, tat mutated, rev mutated, nef mutated; all in-frame
	p2PolTatRevNef.opt.native_C (SEQ ID NO:47)	44	p2 pol, tat native, rev native, nef native; all in-frame
5	p2PolTatRevNef.opt_C (SEQ ID NO:48)	45	p2 Pol, RT mutated, protease non-functional, tat mutated, rev mutated, nef mutated; all in-frame; all in-frame
	protInaRT.YM.opt_C (SEQ ID NO:49)	46	Protease non-functional, RT mutated YM; all in-frame
10	protInaRT.YMWM.opt_C (SEQ ID NO:50)	47	Protease non-functional, RT mutated YM WM; all in-frame
	ProtRT.TatRevNef.opt_C (SEQ ID NO:51)	48	RT mutated, Protease non-functional, tat mutated, rev mutated, nef mutated; all in-frame
	rev.exon1_2.M5-10.opt_C (SEQ ID NO:52)	49	rev exons 1 and 2 mutated; all in-frame
15	tat.exon1_2.opt.C22-37_C (SEQ ID NO:53)	50	tat exons 1 and 2 mutated; all in-frame
	tat.exon1_2.opt.C37_C (SEQ ID NO:54)	51	tat exon 1 and 2 mutated; all in-frame
20	TatRevNef.opt.native_ZA (SEQ ID NO:55)	52	tat native, rev native, nef native; all in-frame
	TatRevNef.opt_ZA (SEQ ID NO:56)	53	tat mutated, rev mutated, nef mutated; all in-frame
	TatRevNefGag C (SEQ ID NO:57)	54	tat mutated, rev mutated, nef mutated, Gag; all in-frame
25	TatRevNefgagCpolIna C (SEQ ID NO:58)	55	tat mutated, rev mutated, nef mutated, Gag complete, pol, RT mutated, protease non-functional; all in-frame

	Name	Figure Number	Description (encoding)
	TatRevNefGagProtInaRTmut C (SEQ ID NO:59)	56	tat mutated, rev mutated, nef mutated, Gag, Protease non-functional, RT mutated; all in-frame
	TatRevNefProtRT opt C (SEQ ID NO:60)	57	tat mutated, rev mutated, nef mutated, protease non-functional, RT mutated; all in-frame
5	gp140modTV1.mut1.dV2 (SEQ ID NO:183)	104	env derived from TV1 mutated in cellular protease cleavage site between gp120/gp41 (may prevent cleavage and may facilitate protein purification) deletion in second variable region (V2)
	gp140modTV1.mut2.dV2 (SEQ ID NO:184)	105	env derived from TV1 mutated in cellular protease cleavage site between gp120/gp41 (may prevent cleavage and may facilitate protein purification) deletion in second variable region (V2)
10	gp140modTV1.mut3.dV2 (SEQ ID NO:185)	106	env derived from TV1 mutated in cellular protease cleavage site between gp120/gp41 (may prevent cleavage and may facilitate protein purification) deletion in second variable region (V2)
	gp140modTV1.mut4.dV2 (SEQ ID NO:186)	107	env derived from TV1 mutated in cellular protease cleavage site between gp120/gp41 (may prevent cleavage and may facilitate protein purification) deletion in second variable region (V2)

Name	Figure Number	Description (encoding)
gp140modTV1.GM161 (SEQ ID NO:187)	108	env derived from TV1 glycosylation site mutation (GM) at amino acid position 161 of Env (N to Q substitution)
gp140modTV1.GM161-195-204 (SEQ ID NO:188)	109	env derived from TV1 glycosylation site mutation (GM) at amino acid positions 161, 195 and 204 of Env (N to Q substitution)
5 gp140modTV1.GM161-204 (SEQ ID NO:189)	110	env derived from TV1 glycosylation site mutation (GM) at amino acid positions 161 and 204 of Env (N to Q substitution)
gp140mod.TV1.GM-V1V2 (SEQ ID NO:190)	111	env derived from TV1 glycosylation site mutation (GM) at various amino acid positions (see also FIG 114)
10 gp140modC8.2mut7.delV2.Kozmod.Ta (SEQ ID NO:191)	112	env derived from TV1 mutated in cellular protease cleavage site between gp120/gp41 (may prevent cleavage and may facilitate protein purification) deletion in second variable region (V2) 5' Kozak sequence and 3' TAAA termination sequence
Nef-myrD124LLAA (SEQ ID NO:203)	115	Nef with mutation in myristoylation site
gp160mod.TV2 (SEQ ID NO:205)	117	env derived from TV2

15 **B. Creating Expression Cassettes Comprising the Synthetic Polynucleotides of the Present Invention.**

The synthetic DNA fragments of the present invention are cloned into the following expression vectors: pCMVKm2, for transient expression assays and DNA

immunization studies, the pCMVKm2 vector was derived from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) and comprises a kanamycin selectable marker, a ColE1 origin of replication, a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a polylinker site was inserted into pCMVKm2 to generate pCMV-link; pESN2dhfr and pCMVPLEdhfr (also known as pCMVIII), for expression in Chinese Hamster Ovary (CHO) cells; and, pAcC13, a shuttle vector for use in the Baculovirus expression system (pAcC13, was derived from pAcC12 which was described by Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990). See, also co-owned WO 00/39303, WO 00/39302, WO 00/39304, WO 02/04493, for a description of these vectors.

Briefly, construction of pCMVPLEdhfr (pCMVIII) was as follows. To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site) leader was PCR-amplified from pCite-4a+ (Novagen, Inc., Milwaukee, WI) and inserted into pET-23d (Novagen, Inc., Milwaukee, WI) as an *Xba*-*Nco* fragment to give pET-EMCV. The *dhfr* gene was PCR-amplified from pESN2dhfr to give a product with a Gly-Gly-Gly-Ser spacer in place of the translation stop codon and inserted as an *Nco*-*Bam*H1 fragment to give pET-E-DHFR. Next, the attenuated *neo* gene was PCR amplified from a pSV2Neo (Clontech, Palo Alto, CA) derivative and inserted into the unique *Bam*H1 site of pET-E-DHFR to give pET-E-DHFR/Neo_(m2). Then, the bovine growth hormone terminator from pCDNA3 (Invitrogen, Inc., Carlsbad, CA) was inserted downstream of the *neo* gene to give pET-E-DHFR/Neo_(m2)BGHt. The EMCV-*dhfr/neo* selectable marker cassette fragment was prepared by cleavage of pET-E-DHFR/Neo_(m2)BGHt. The CMV enhancer/promoter plus Intron A was transferred from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) as a *Hind*III-*Sal*I fragment into pUC19 (New England Biolabs, Inc., Beverly, MA). The vector backbone of pUC19 was deleted from the *Nde*I to the *Sap*I sites. The above described DHFR cassette was added to the construct such that the EMCV IRES followed the CMV promoter to produce the final construct. The vector also contained an *amp^r* gene and an SV40 origin of replication.

Expression vectors of the present invention contain one or more of the synthetic coding sequences disclosed herein, e.g., shown in the Figures. When the expression cassette contains more than one coding sequence the coding sequences may all be in-frame to generate one polypeptide; alternately, the more than one polypeptide coding sequences may comprise a polycistronic message where, for example, an IRES is placed 5' to each polypeptide coding sequence.

Example 2

Expression Assays for the

Synthetic Coding Sequences

The wild-type sequences are cloned into expression vectors having the same features as the vectors into which the synthetic HIV-derived sequences were cloned.

Expression efficiencies for various vectors carrying the wild-type (any known isolated) and corresponding synthetic sequence(s) are evaluated as follows. Cells from several mammalian cell lines (293, RD, COS-7, and CHO; all obtained from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) are transfected with 2 µg of DNA in transfection reagent LT1 (PanVera Corporation, 545 Science Dr., Madison, WI). The cells are incubated for 5 hours in reduced serum medium (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium is then replaced with normal medium as follows: 293 cells, IMDM, 10% fetal calf serum, 2% glutamine (BioWhittaker, Walkersville, MD); RD and COS-7 cells, D-MEM, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD); and CHO cells, Ham's F-12, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The cells are incubated for either 48 or 60 hours. Supernatants are harvested and filtered through 0.45 µm syringe filters and, optionally, stored at -20°C.

Supernatants are evaluated using the Coulter p24-assay (Coulter Corporation, Hialeah, FL, US), using 96-well plates coated with a suitable monoclonal antibody directed against an HIV antigen (e.g. a murine monoclonal directed against an HIV core antigen). The appropriate HIV antigen binds to the coated wells and biotinylated antibodies against HIV recognize the bound antigen. Conjugated streptavidin-

horseradish peroxidase reacts with the biotin. Color develops from the reaction of peroxidase with TMB substrate. The reaction is terminated by addition of 4N H₂SO₄. The intensity of the color is directly proportional to the amount of HIV antigen in a sample.

5 Chinese hamster ovary (CHO) cells are also transfected with plasmid DNA encoding the synthetic HIV polypeptides described herein (*e.g.*, pESN2dhfr or pCMVIII vector backbone) using Mirus TransIT-LT1 polyamine transfection reagent (Pan Vera) according to the manufacturers instructions and incubated for 96 hours. After 96 hours, media is changed to selective media (F12 special with 250 µg/ml
10 G418) and cells are split 1:5 and incubated for an additional 48 hours. Media is changed every 5-7 days until colonies start forming at which time the colonies are picked, plated into 96 well plates and screened by Capture ELISA. Positive clones are expanded in 24 well plates and are screened several times for HIV protein production by Capture ELISA, as described above. After reaching confluency in 24 well plates,
15 positive clones are expanded to T25 flasks (Corning, Corning, NY). These are screened several times after confluency and positive clones are expanded to T75 flasks.

Positive T75 clones are frozen in LN₂ and the highest expressing clones are amplified with 0-5 µM methotrexate (MTX) at several concentrations and plated in 100mm culture dishes. Plates are screened for colony formation and all positive clones
20 are again expanded as described above. Clones are expanded and amplified and screened at each step capture ELISA. Positive clones are frozen at each methotrexate level. Highest producing clones are grown in perfusion bioreactors (3L, 100L) for expansion and adaptation to low serum suspension culture conditions for scale-up to larger bioreactors.

25 Data from experiments performed in support of the present invention show that the synthetic HIV expression cassettes provided dramatic increases in production of their protein products, relative to the native (wild-type) sequences, when expressed in a variety of cell lines and that stably transfected CHO cell lines, which express the desired HIV polypeptide(s), may be produced. Production of HIV polypeptides using
30 CHO cells provides (i) correct glycosylation patterns and protein conformation (as determined by binding to panel of MAbs); (ii) correct binding to CD4 receptor

molecules; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification.

Example 3

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Western Blot Analysis of Expression

Western blot analysis of cells transfected with the HIV expression cassettes described herein are performed essentially as described in co-owned WO 00/39302. Briefly, human 293 cells are transfected as described in Example 2 with pCMV6a-based vectors containing native or synthetic HIV expression cassettes. Cells are
10 cultivated for 60 hours post-transfection. Supernatants are prepared as described. Cell lysates are prepared as follows. The cells are washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego, CA) are loaded with 20 μ l of
15 supernatant or 12.5 μ l of cell lysate. A protein standard is also loaded (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA). Electrophoresis is carried out and the proteins are transferred using a BioRad Transfer Chamber (BioRad Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where
20 the transfer is performed at 100 volts for 90 minutes. The membranes are exposed to HIV-1-positive human patient serum and immunostained using o-phenylenediamine dihydrochloride (OPD; Sigma).

The results of the immunoblotting analysis are used to show that cells containing the synthetic HIV expression cassette produce the expected HIV-
25 polypeptide(s) at higher per-cell concentrations than cells containing the native expression cassette.

Example 4In Vivo Immunogenicity of Synthetic HIV Expression CassettesA. Immunization

To evaluate the immunogenicity of the synthetic HIV expression cassettes, a mouse study may be performed. The plasmid DNA, e.g., pCMVKM2 carrying an expression cassette comprising a synthetic sequence of the present invention, is diluted to the following final concentrations in a total injection volume of 100 μ l: 20 μ g, 2 μ g, 0.2 μ g, and 0.02 μ g. To overcome possible negative dilution effects of the diluted DNA, the total DNA concentration in each sample is brought up to 20 μ g using the vector (pCMVKM2) alone. As a control, plasmid DNA comprising an expression cassette encoding the native, corresponding polypeptide is handled in the same manner. Twelve groups of four Balb/c mice (Charles River, Boston, MA) are intramuscularly immunized (50 μ l per leg, intramuscular injection into the *tibialis anterior*) using varying dosages.

B. Humoral Immune Response

The humoral immune response is checked with a suitable anti-HIV antibody ELISAs (enzyme-linked immunosorbent assays) of the mice sera 0 and 4 weeks post immunization (groups 5-12) and, in addition, 6 and 8 weeks post immunization, respectively, 2 and 4 weeks post second immunization (groups 1-4).

The antibody titers of the sera are determined by anti-HIV antibody ELISA. Briefly, sera from immunized mice were screened for antibodies directed against an appropriate HIV protein (e.g., HIV p55 for Gag). ELISA microtiter plates are coated with 0.2 μ g of HIV protein per well overnight and washed four times; subsequently, blocking is done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum is added. Sera are tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates are washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates are washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well is

measured after 15 minutes. The titers reported are the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.).

The results of the mouse immunizations with plasmid-DNAs are used to show that the synthetic expression cassettes provide improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization induces a secondary immune response after two weeks (groups 1-3).

C. Cellular Immune Response

The frequency of specific cytotoxic T-lymphocytes (CTL) is evaluated by a standard chromium release assay of peptide pulsed Balb/c mouse CD4 cells. HIV protein-expressing vaccinia virus infected CD-8 cells are used as a positive control (vv-protein). Briefly, spleen cells (Effector cells, E) are obtained from the BALB/c mice (immunized as described above). The cells are cultured, restimulated, and assayed for CTL activity against, e.g., Gag peptide-pulsed target cells as described (Doe, B., and Walker, C.M., *AIDS* 10(7):793-794, 1996). Cytotoxic activity is measured in a standard ^{51}Cr release assay. Target (T) cells are cultured with effector (E) cells at various E:T ratios for 4 hours and the average cpm from duplicate wells is used to calculate percent specific ^{51}Cr release.

Cytotoxic T-cell (CTL) activity is measured in splenocytes recovered from the mice immunized with HIV DNA constructs described herein. Effector cells from the DNA-immunized animals exhibit specific lysis of HIV peptide-pulsed SV-BALB (MHC matched) targets cells indicative of a CTL response. Target cells that are peptide-pulsed and derived from an MHC-unmatched mouse strain (MC57) are not lysed. The results of the CTL assays are used to show increased potency of synthetic HIV expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

Example 5

In Vivo Immunogenicity of Synthetic HIV Expression Cassettes

A. General Immunization Methods

To evaluate the immunogenicity of the synthetic HIV expression cassettes, studies using guinea pigs, rabbits, mice, rhesus macaques and baboons are performed.

The studies are typically structured as follows: DNA immunization alone (single or multiple); DNA immunization followed by protein immunization (boost); DNA immunization followed by Sindbis particle immunization; immunization by Sindbis particles alone.

5

B. Guinea Pigs

Experiments may be performed using guinea pigs as follows. Groups comprising six guinea pigs each are immunized intramuscularly or mucosally at 0, 4, and 12 weeks with plasmid DNAs encoding expression cassettes comprising one or more the sequences described herein. The animals are subsequently boosted at approximately 18 weeks with a single dose (intramuscular, intradermally or mucosally) of the HIV protein encoded by the sequence(s) of the plasmid boost and/or other HIV proteins. Antibody titers (geometric mean titers) are measured at two weeks following the third DNA immunization and at two weeks after the protein boost. These results are used to demonstrate the usefulness of the synthetic constructs to generate immune responses, as well as, the advantage of providing a protein boost to enhance the immune response following DNA immunization.

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C. Rabbits

Experiments may be performed using rabbits as follows. Rabbits are immunized intramuscularly, mucosally, or intradermally (using a Bioject needleless syringe) with plasmid DNAs encoding the HIV proteins described herein. The nucleic acid immunizations are followed by protein boosting after the initial immunization. Typically, constructs comprising the synthetic HIV-polypeptide-encoding polynucleotides of the present invention are highly immunogenic and generate substantial antigen binding antibody responses after only 2 immunizations in rabbits.

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D. Humoral Immune Response

In any immunized animal model, the humoral immune response is checked in serum specimens from the immunized animals with an anti-HIV antibody ELISAs (enzyme-linked immunosorbent assays) at various times post-immunization. The

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antibody titers of the sera are determined by anti-HIV antibody ELISA as described above. Briefly, sera from immunized animals are screened for antibodies directed against the HIV polypeptide/protein(s) encoded by the DNA and/or polypeptide used to immunize the animals. Wells of ELISA microtiter plates are coated overnight with the selected *HIV* polypeptide/protein and washed four times; subsequently, blocking is done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum is added. Sera are tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates are washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates are washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well is measured after 15 minutes. Titers are typically reported as the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.).

Cellular immune response may also be evaluated.

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Example 6

DNA-immunization of Baboons and Rhesus Macaques Using Expression Cassettes Comprising the Synthetic HIV Polynucleotides of the Present Invention

A. Baboons

20 Four baboons are immunized 3 times (weeks 0, 4 and 8) bilaterally, intramuscular into the quadriceps or mucosally using the gene delivery vehicles described herein. The animals are bled two weeks after each immunization and an HIV antibody ELISA is performed with isolated plasma. The ELISA is performed essentially as described above except the second antibody-conjugate is an anti-human
25 IgG, g-chain specific, peroxidase conjugate (Sigma Chemical Co., St. Louis, MD 63178) used at a dilution of 1:500. Fifty μ g/ml yeast extract may be added to the dilutions of plasma samples and antibody conjugate to reduce non-specific background due to preexisting yeast antibodies in the baboons. Lymphoproliferative responses to are observed in baboons two weeks post-fourth immunization (at week 14), and
30 enhanced substantially post-boosting with HIV-polypeptide (at week 44 and 76). Such proliferation results are indicative of induction of T-helper cell functions.

B. Rhesus Macaques

The improved potency of the synthetic, codon-modified *HIV*-polypeptide encoding polynucleotides of the present invention, when constructed into expression plasmids may be confirmed in rhesus macaques. Typically, the macaques have
5 detectable *HIV*-specific CTL after two or three 1 mg doses of modified *HIV* polynucleotide. In sum, these results demonstrate that the synthetic *HIV* DNA is immunogenic in non-human primates. Neutralizing antibodies may also be detected.

Example 7

10 Co-Transfection of Monocistronic and Multicistronic Constructs

The present invention includes co-transfection with multiple, monocistronic expression cassettes, as well as, co-transfection with one or more multi-cistronic expression cassettes, or combinations thereof.

Such constructs, in a variety of combinations, may be transfected into 293T
15 cells for transient transfection studies.

For example, a bicistronic construct may be made where the coding sequences for the different *HIV* polypeptides are under the control of a single CMV promoter and, between the two coding sequences, an IRES (internal ribosome entry site (EMCV IRES); Kozak, M., Critical Reviews in Biochemistry and Molecular Biology
20 27(45):385-402, 1992; Witherell, G.W., et al., Virology 214:660-663, 1995) sequence is introduced after the first *HIV* coding sequence and before the second *HIV* coding sequence.

Supernatants collected from cell culture are tested for the presence of the *HIV* proteins and indicate that appropriate proteins are expressed in the transfected cells
25 (e.g., if an *Env* coding sequence was present the corresponding *Env* protein was detected; if a *Gag* coding sequence was present the corresponding *Gag* protein was detected, etc).

The production of chimeric VLPs by these cell lines may be determined using electron microscopic analysis. (See, e.g., co-owned WO 00/39302).

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Example 8Accessory gene components for an HIV-1 vaccine: functional analysis of mutated Tat,
Rev and Nef Type C antigens

The HIV-1 regulatory and accessory genes have received increased attention as
5 components of HIV vaccines due to their role in viral pathogenesis, the high ratio of
highly conserved CTL epitopes and their early expression in the viral life cycle.
Because of various undesirable properties of these genes, questions regarding their
safety and suitability as vaccine components have been raised. Experiments performed
in support of the present invention have analyzed candidate HIV-1 subtype C *tat*, *rev*,
10 and *nef* mutants for efficient expression and inactivation of potential deleterious
functions. Other HIV subtype accessory genes may be evaluated similarly.

Sequence-modified, mutant *tat*, *rev*, and *nef* genes coding for consensus Tat,
Rev and Nef proteins of South African HIV-1 subtype C were constructed using
overlapping synthetic oligonucleotides and PCR-based site-directed mutagenesis.
15 Constructs of the wild-type genes of the isolates closely resembling the respective
consensus sequences were also made by PCR. *In vitro* expression of the constructs
was analyzed by western blotting. The *trans*-activation activity of the Tat mutants and
nuclear RNA export activity of the Rev mutants were studied after transfection of
various cell lines using reporter-gene-based functionality assays.

20 *In vitro* expression of all constructs was demonstrated by western blotting
using antigen specific mouse serum generated by DNA vaccination of mice with Tat,
Rev, or Nef-expression plasmids. Expression levels of the sequence-modified genes
were significantly higher than the wild-type genes.

Subtype B and C Tat cDNA was mutated to get TatC22, TatC37, and
25 TatC22/37. Tat activity assays in three cell lines (RD, HeLa and 293). In the
background of the subtype C consensus Tat, a single mutation at C22 was insufficient
to inactivate LTR-dependent CAT expression. In contrast, this activity was
significantly impaired in RD, 293 and HeLa cells using the single mutation, C37, or the
double mutation, C22C37 (see Table B). Corresponding results were obtained for Tat
30 mutants derived from subtype B strains.

Exemplary results are presented in Figure 4 for transactivation activity of Tat mutants on LTR-CAT plasmid in 293 cells. Three independent assays were performed for each construct (Figure 4, legend (1), (2), (3)).

The subtype C constructs TatC22ProtRTTatRevNef and
 5 ProtRTTatC22RevNef showed reduced Tat activity when compared to TatC22 alone, probably due to structural changes caused by the fusion protein.

For Rev constructs, to test for the loss of function, a CAT assay with a reporter plasmid including native or mutated Rev was used. As shown in Figure 5, compared to wild-type Rev, the mRNA export function of the subtype C Rev with a
 10 double mutation, M5M10 (see Table B), was significantly lower. The background levels are shown in the "mock" data and the pDM128 reporter plasmid without Rev data. Two independent assays were performed for each construct (Figure 5, legend (1), (2)).

Assays to measure Nef-specific functions may also be performed (Nef
 15 mutations are described in Table B). For example, FACs analysis is used to look for the presence of MHC1 and CD4 on cell surfaces. Cells are assayed in the presence and absence of Nef expression (for controls), as well as using the synthetic polynucleotides of the present invention that encode native nef protein and mutated nef protein. Down-regulation of MHC1 and CD4 expression indicates that the nef gene
 20 product is not functional, i.e., if nef is non-functional there is no down regulation.

These data demonstrate the impaired functionality of *tat* and *rev* DNA immunogens that may form part of a multi-component HIV-1 subtype C vaccine. In contrast to previous published data by other groups, the C22 mutation did not
 25 sufficiently inactivate the transactivation function of Tat. The C37 mutation appeared to be required for inactivation of subtype C and subtype B Tat proteins.

Example 9

Evaluation of immunogenicity of various HIV polypeptide encoding plasmids

As noted above, the immunogenicity of any of the polynucleotides or
 30 expression cassettes described herein is readily evaluated. In the following table (Table D) are exemplified procedures involving a comparison of the immunogenicity of

subtype B and C envelope plasmids, both individually and as a mixed-subtype vaccine, using electroporation, in rabbits. It will be apparent that such methods are equally applicable to any other HIV polypeptide.

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Table D

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Grp	Animal	Imm'n #	Adjuvant	Immunogen	Total Dose	Vol/ Site	Sites/ Animal	Route
1	1-4	1, 2	-	pCMV 160 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 160 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
2	5-8	1, 2	-	pCMV 160 dV2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 160 dV2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
3	9-12	1, 2	-	pCMV 160 dV1/V2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 160 dV1/V2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
4	13-16	1, 2	-	pCMV 140 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 140 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
5	17-20	1, 2	-	pCMV140dV2TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)

Grp	Animal	Imm'n #	Adjuvant	Immunogen	Total Dose	Vol/ Site	Sites/ Animal	Route
5		3	-	pCMV140dV2TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
	6	1, 2	-	pCMV 140 dV1/V2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 140 dV1/V2 TV1 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
	7	1, 2	-	pSIN140dV2SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pSIN 140 dV2 SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
	8	1, 2	-	pCMV 140 dV2 SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 140 dV2 SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut
	9	1, 2	-	pCMV 140 Q154 SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
		3	-	pCMV 140 Q154 SF162 DNA	1.0mg	0.5ml	2	IM/Quad (Electro)
			MF59C	Protein TBD	0.05mg	0.5ml	2	IM/Glut

10	37-40	1, 2	-	pCMV 140 dV2 SF162 DNA pCMV 140 dV2 TV1 DNA	1.0mg 1.0mg	0.5ml	2	IM/Quad (Electro)			
		3	-	pCMV 140 dV2 SF162 DNA pCMV 140 dV2 TV1 DNA	1.0mg 1.0mg						
		MF59C	Protein TBD	0.05mg	0.5ml				2	IM/Glut	
11	41-44	1, 2	-	pCMV 140 dV2 SF162 DNA pCMV 140 dV2 TV1 DNA	1.0mg 1.0mg	0.5ml	2	IM/Quad (Electro)			
		1, 2	-	pCMV 140 dV2 SF162 DNA pCMV 140 dV2 TV1 DNA	1.0mg 1.0mg				0.5ml	2	IM/Quad (Electro)
		3	MF59C	Protein TBD	0.05mg						

The MF59C adjuvant is a microfluidized emulsion containing 5% squalene,

10 0.5% Tween 80, 0.5% span 85, in 10mM citrate pH 6, stored in 10mL aliquots at 4°C.

Immunogens are prepared as described in the following table (Table E) for administration to animals in the various groups. Concentrations may vary from those described in the table, for example depending on the sequences and/or proteins being used.

15 Table E

Group	Preparation
1-9	Immunization 1-3: pCMV and pSIN based plasmid DNA in Saline + Electroporation Subtype B and C plasmids will be provided frozen at a concentration of 1.0mg/ml in sterile 0.9% saline. Store at -80°C until use. Thaw DNA at room

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Group	Preparation
5	<p>temperature; the material should be clear or slightly opaque, with no particulate matter. Animals will be shaved prior to immunization, under sedation of 1x dose IP (by animal weight) of Ketamine-Xylazine (80mg/ml - 4mg/ml). Immunize each rabbit with 0.5ml DNA mixture per side (IM/Quadriceps), 1.0ml per animal. Follow the DNA injection with Electroporation using a 6-needle circular array with 1cm diameter, 1cm needle length. Electroporation pulses were given at 20V/mm, 50ms pulse length, 1 pulse/s.</p>
10	<p>Immunization 3: Protein Immunization Proteins will be provided at 0.1mg/ml in citrate buffer. Store at -80°C until use. Thaw at room temperature; material should be clear with no particulate matter. Add equal volume of MF59C adjuvant to thawed protein and mix well by inverting the tube. Immunize each rabbit with 0.5ml adjuvanted protein per side, IM/Glut for a total of 1.0ml per animal. Use material within 1 hour of the addition of adjuvant.</p>
15	<p>Immunization 1-3: Combined subtype B and C plasmid DNA in Saline The immunogen will be provided at 2.0mg/ml total DNA (1mg/ml of each plasmid) in sterile 0.9% saline. Store at -80°C until use. Thaw DNA at room temperature; the material should be clear or slightly opaque, with no particulate matter. Animals will be shaved prior to immunization, under sedation of 1x dose IP (by animal weight) of Ketamine-Xylazine (80mg/ml - 4mg/ml). Immunize each rabbit with 0.5ml DNA mixture per side (IM/Quadriceps), 1.0ml per animal. Follow the DNA injection with Electroporation using a 6-needle circular array with 1cm diameter, 1cm needle length. Electroporation pulses were given at 20V/mm, 50ms pulse length, 1 pulse/s.</p>
10-11	<p>Immunization 3: Protein Immunization Proteins will be provided at 0.1mg/ml in citrate buffer. Store at -80°C until use. Thaw at room temperature; material should be clear with no particulate matter. Add equal volume of MF59C adjuvant to thawed protein and mix well by inverting the tube. Immunize each rabbit with 0.5ml adjuvanted protein per side, IM/Glut for a total of 1.0ml per animal. Use material within 1 hour of the addition of adjuvant.</p>
20	

The immunization (Table F) and bleeding (Table G) schedules are as follows:

18133.003

Table F

Imm'n: Weeks: Group	1 0	2 4	3 16	3 16
1	pCMV 160 TV1 DNA	pCMV 160 TV1 DNA	pCMV 160 TV1 DNA	Protein + MF59C
2	pCMV 160 dV2 TV1 DNA	pCMV 160 dV2 TV1 DNA	pCMV 160 dV2 TV1 DNA	Protein + MF59C
3	pCMV 160 dV1/N2 TV1 DNA	pCMV 160 dV1/N2 TV1 DNA	pCMV 160 dV1/N2 TV1 DNA	Protein + MF59C
4	pCMV 140 TV1 DNA	pCMV 140 TV1 DNA	pCMV 140 TV1 DNA	Protein + MF59C
5	pCMV 140 dV2 TV1 DNA	pCMV 140 dV2 TV1 DNA	pCMV 140 dV2 TV1 DNA	Protein + MF59C
6	pCMV 140 dV1/N2 TV1 DNA	pCMV 140 dV1/N2 TV1 DNA	pCMV 140 dV1/N2 TV1 DNA	Protein + MF59C
7	pSIN 140 dV2 SF162 DNA	pSIN 140 dV2 SF162 DNA	pSIN 140 dV2 SF162 DNA	Protein + MF59C
8	pCMV 140 dV2 SF162 DNA	pCMV 140 dV2 SF162 DNA	pCMV 140 dV2 SF162 DNA	Protein + MF59C
9	pCMV 140 Q154 SF162 DNA	pCMV 140 Q154 SF162 DNA	pCMV 140 Q154 SF162 DNA	Protein + MF59C
10	pCMV 140 dV2 SF162 DNA +	pCMV 140 dV2 SF162 DNA +	pCMV 140 dV2 SF162 DNA +	Protein + MF59C
11	pCMV 140 dV2 TV1 DNA	pCMV 140 dV2 TV1 DNA	pCMV 140 dV2 TV1 DNA	Protein + MF59C
	pCMV 140 dV2 SF162 DNA +	pCMV 140 dV2 SF162 DNA +	pCMV 140 dV2 SF162 DNA +	Protein + MF59C
	pCMV 140 dV1/N2 TV1 DNA	pCMV 140 dV1/N2 TV1 DNA	pCMV 140 dV1/N2 TV1 DNA	Protein + MF59C

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Table G

Bleed:	0	1	2	3	4	5	6	7	8	9	10
Week:	-3	4	6	8	12	16	18	20	24	28	TBD
Sample:	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum	Clotted Bld. for Serum
Volume:	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each	20cc each
Method:	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	AA/MEV	CP

20

Example 10

Mice Immunization Studies with Gag and Pol Constructs

Cellular and Humoral immune responses were evaluated in mice (essentially as described in Example 4) for the following constructs: Gag, GagProtease(+FS) (GP1, protease codon optimized and inactivation of INS; GP2, protease only inactivation of INS), GagPol Δ integrase with frameshift (gagFSpol), and GagPol Δ integrase in-frame (GagPol) (see Figure 118). Versions of GagPol Δ integrase in-frame were also designed with attenuated (GagPolAtt) or non-functional Protease (GagPolIna).

In vitro expression data showed comparable expression of p55Gag and p66RT using Gag alone, GagProtease(+FS), gagFSpol and GagPolIna. Constructs with fully functional or attenuated protease (GagPol or GagPolAtt) were less efficient in expression of p55Gag and p66RT, possibly due to cytotoxic effects of protease.

DNA immunization of mice using Gag vs. GP1 and GP2 in pCMV vectors was performed intramuscularly in the tibialis anterior. Mice were immunized at the start of the study (0 week) and 4 weeks later. Bleeds were performed at 0, 4, and 6 weeks. DNA doses used were as follows: 20 μ g, 2 μ g, 0.2 μ g, and 0.02 μ g.

DNA immunization of mice using Gag vs. gagFSpol in pCMV vectors was performed intramuscularly in the tibialis anterior. Mice were immunized at the start of the study (0 week) and challenged 4 weeks later with recombinant vaccinia virus encoding Gag (rVVgag). Bleeds were performed at 0 and 4 weeks. DNA doses used were as follows: 20 μ g, 2 μ g, 0.2 μ g, and 0.02 μ g.

DNA immunization of mice using Gag vs. gagFSpol and gagpol in pCMV vectors was performed intramuscularly in the tibialis anterior. Mice were immunized at the start of the study (0 week) and challenged 4 weeks later with recombinant vaccinia virus encoding Gag (rVVgag). Bleeds were performed at 0 and 4 weeks. DNA doses used were as follows: 2 μ g, 0.2 μ g, 0.02 μ g, and 0.002 μ g.

Cellular immune responses against Gag were comparable for all tested variants, for example, Gag, GagProtease, gagFSpol and GagPolIna all had comparable potencies.

Humoral immune responses to Gag were also comparable with the exception of GP2 and especially GP1. Humoral immune responses were weaker in constructs

comprising functional or attenuated proteases which may be due to less efficient secretion of p55Gag caused by overactive protease.

In vitro and in vivo experiments, performed in support of the present invention, suggest that the expression and immunogenicity of Gag was comparable with all
5 constructs. Exceptions were GagPol in-frame with fully functional or attenuated protease. This may be the result of cytotoxic effects of protease. The immune response in mice correlated with relative levels of expression in vitro.

Example 11

10 Protein Expression, Immunogenicity, and Generation of Neutralizing Antibodies Using Type C Derived Envelope Polypeptides

Envelope (Env) vaccines derived from the subtype C primary isolate, TV1, recovered from a South African individual, were tested in rabbits as follows. Gene cassettes were designed to express the gp120 (surface antigen), gp140 (surface antigen
15 plus ectodomain of transmembrane protein, gp41), and full-length (gp120 plus gp41) gp160 forms of the HIV-1 envelope polypeptide with and without deletions of the variable loop regions, V2 and V1V2. All of the genes were sequence-modified to enhance expression of the encoded Env glycoproteins in a Rev-independent fashion and they were subsequently cloned into pCMV-based plasmid vectors for DNA
20 vaccine and protein production applications as described above. The sequences were codon optimized as described herein. Briefly, all the modified envelope genes were cloned into the Chiron pCMVlink plasmid vector, preferably into EcoRI/XhoI sites.

A. Protein Expression

25 Full-length (gp160), truncated gp140 (Env ectodomain only) and gp120 native versions of the TV1 Env antigen were produced from the expression cassettes described herein. The gp140 encoding sequences were transiently transfected into 293T cells. The expression levels of the gene products were evaluated by an in-house antigen capture ELISA. Envelope genes constructed from the native sequences of
30 TV001c8.2, TV001c8.5 and TV002c12.1 expressed the correct proteins in vitro, with gp140TV001c8.2 exhibiting the highest level of expression. In addition, the Env

protein expressed from the TV1-derived clone 8.2 was found to bind the CD4 receptor protein indicating that this feature of the expressed protein is maintained in a functional conformation. The receptor binding properties/functionality of the expressed TV1 gp160 protein result was also confirmed by a cell-fusion assay.

5 Total expression increased approximately 10-fold for synthetic gp140 constructs compared with the native gp140 gene cassettes. Both the modified gp120 and gp140 variants secreted high amounts of protein in the supernatant. In addition, the V2 and V1V2 deleted forms of gp140 expressed approximately 2-fold more protein than the intact gp140. Overall, the expression levels of synthetic gp140 gene
10 variants increased 10 to 26-fold compared with the gp140 gene with native sequences.

In sum, each synthetic construct tested showed more than 10-fold increased levels of expression relative to those using the native coding sequences. Moreover, all expressed proteins were of the expected molecular weights and were shown to bind CD4. Stable CHO cell lines were derived and small-scale protein purification methods
15 were used to produce small quantities of each of the undeleted and V-deleted oligomeric forms (o-gp140) of these proteins for vaccine studies.

B. Neutralization properties of TV001 and TV002 viral isolates

The transient expression experiment showed that the envelope genes derived
20 from the TV001 and TV002 virus isolates expressed the desired protein products. Relative neutralization sensitivities of these two viral strains using sera from 18 infected South African individuals (subtypes B and C) were as follows. At a 1:10 serum dilution, the TV2 strain was neutralized by 18 of 18 sera; at 1:50, 16 of 18; at 1:250, 15/18. In comparison, the TV1 isolate was neutralized by 15 of 18 at 1:10;
25 only 6 of 18 at 1:50; and none of the specimens at 1:250. In addition, the TV001 patient serum showed neutralization activity against the TV002 isolate at all dilutions tested. In contrast, the TV002 showed neutralization of TV001 only at the 1:10 serum dilution. These results suggest that TV001 isolate is capable of inducing a broader and more potent neutralizing antibody response in its infected host than TV002.

30

C. Immunogenicity of the modified TV1 Env DNA and protein antigens in rabbit studies

TV1 Env DNA (comprising the synthetic expression cassettes) and protein vaccines were administered as shown in the following Table H.

Table H

Groups	Plasmid DNA (0, 4, and 20 wks)	Protein boost (20 wks)
1	pCMVgp160.TV1	o-gp140.TV1
2	pCMVgp160dV2.TV1	o-gp140dV2.TV1
3	pCMVgp160dV1V2.TV1	o-gp140dV1V2.TV1
4	pCMVgp140.TV1	o-gp140.TV1
5	pCMVgp140dV2.TV1	o-gp140dV2.TV1
6	pCMVgp140dV1V2.TV1	o-gp140dV1V2.TV1
7	pCMVgp140dV2.SF162	o-gp140dV2.SF162

Seven groups of 4 rabbits per group were immunized with the designated plasmid DNA and oligomeric Env protein antigens. Three doses of DNA, 1mg of DNA per animal per immunization, were administered intramuscularly by needle injection followed by electroporation on weeks 0, 4, and 20 weeks. A single dose of 100 ug of Env protein in MF59 adjuvant also was given intramuscularly in a separate site at 20 weeks.

The DNA immunization used subtype C sequence-modified genes (TV1) -- gp160, gp160dV2, gp160dV1V2, gp140, gp140dV2 and gp140dV1V2 -- as well as a subtype B SF162 sequence modified gp140dV2. DNA immunizations were performed at 0, 4, and 20 weeks by needle injection by the intramuscular route using electroporation to facilitate transfection of the muscle cells and of resident antigen presenting cells.

A single Env protein booster (in MF59 adjuvant) was given at 20 weeks by intramuscular injection at a separate site. Antibody titers were evaluated by ELISA following each successive immunization. Serum specimens were collected at 0, 4, 6, 8, 12, 22, and 24 weeks. Serum antibody titers were measured on ELISA. 96-well plates were coated with a protein in a concentration of 1ug/ml. Serum samples were diluted serially 3-fold. Goat anti-rabbit peroxidase conjugate (1:20,000) was used for

detection. TMB was used as the substrate, and the antibody titers were read at 0.6 OD at 450nm.

Neutralizing antibody responses against PBMC-grown R5 HIV-1 strains were monitored in the sera collected from the immunized rabbits using two different assays in two different laboratories, the 5.25 reporter cell-line based assay at Chiron and the PBMC-based assay of David Montefiori at Duke University. Results are shown in Figures 121, 122, and 123. The Chiron assay was conducted essentially as follows. Neutralizing antibody responses against the PBMC-grown subtype C TV001 and TV002 strains were measured using an in-house reporter cell line assay that uses the 5.25 cell line. This cell has CD4, CCR5, CXCR4 and BONZO receptor/co-receptors on its cell membrane. The parental CEM cell line was derived from a 4-year-old Caucasian female with acute lymphoblastic leukemia, which was fused with the human B cell line 721.174, creating CEMx174. LTR-GFP was transfected into the cells after the CCR5 gene (about 1.1 kb) was cloned into the BamH-I (5') and Sal-I (3') of the pBABE puro retroviral vector, and subsequently introduced into the CEMx174. The green fluorescence protein (GFP) of the cells was detected by flow cytometer (FACScan). For the virus neutralization assay, 50 ul of titrated virus and 50 ul of diluted immune or pre-immune serum were incubated at room temperature for one hour. This mixture was added into wells with 10^4 /ml cells plated in a 24 well plate, and incubated at 37°C for 5 to 7 days. The cells were then fixed with 2% of formaldehyde after washing with PBS. Fifteen thousand events (cells) were collected for each sample on a Becton Dickinson FACScan using Cellquest software. The data presented were the mean of the triplicate wells. The percent neutralization was calculated compared to the virus control using the following equation: % virus Inhibition = (virus control - experimental)/(virus control - cell control) x 100. Any virus inhibition observed in the pre-bleed has been subtracted for each individual animal. Values >50% are considered positive and are highlighted in gray.

In Figure 122, the “#” indicates that animals had high levels of virus inhibition in pre-bleed serum (>20% virus inhibition) that impacted the magnitude of the observed inhibition and in some cases, our ability to score the serum as a positive or negative for the presence of significant neutralizing antibody activity (< 50%)

inhibition).

For the data presented in Figure 123, serum samples were collected after a single protein boost (post-third) were screened in triplicate at a 1:8 dilution with virus (1:24 after addition of cells). Values shown are the % reduction in p24 synthesis relative to that in the corresponding pre-bleed control samples. Zero values indicate no or negative values were measured. NV, not valid due to virus inhibition in pre-immune serum. Neutralization was considered positive when p24 was reduced by at least 80%; these samples are highlighted in dark gray. Sample with lighter gray shading showed at least a 50% reduction in p24 synthesis.

Figure 119 shows the ELISA data when plates were coated with the monomeric gp120.TV1 protein. This protein is homologous to the subtype C genes used for the immunization. All immunization groups produced high antibody titers after the second DNA immunization. The groups immunized with gp140 forms of DNA have relatively higher geometric mean antibody titers as compared to the groups using gp160 forms after both first and second DNA immunizations. Both the gp140.TV1 and gp140dV1V2.TV1 genes produced high antibody titers at about 10^4 at two weeks post second DNA; the gp140dV2.TV1 plasmid yielded the highest titers of antibodies ($>10^4$) at this time point and all others.. The binding antibody titers to the gp120.TV1 protein were higher for the group immunized with the homologous gp140dV2.TV1 genes than that with the heterologous gp140dV2.SF162 gene which showed titers of about 10^3 . All the groups, showed some decline in antibody titers by 8 weeks post the second DNA immunization. Following the DNA plus protein booster at 20 weeks, all groups reached titers above that previously observed after the second DNA immunization (0.5 – 1.0 log increases were observed). After the protein boost, all animals receiving the o-gp140dV2.TV1 protein whether primed by the gp140dV2.TV1 or gp160dV2.TV1 DNA, showed the highest Ab titers.

Binding antibody titers were also measured using ELISA plates coated with either oligomeric subtype C o-gp140dV2.TV1 or subtype B o-gp140dV2.SF162 proteins (Figure 120). For all the TV1 Env immunized groups, the antibody titers measured using the oligomeric protein, o-gp140dV2.TV1 were higher than those measured using the monomeric (non-V2-deleted) protein, gp120.TV1. In fact, for

these groups, the titers observed with the heterologous subtype B o-gp140dV2.SF162 protein were comparable to or greater than those measured with the subtype C TV1 gp120. Nevertheless, all groups immunized with subtype C immunogens showed higher titers binding to the subtype C o-gp140dV2.TV1 protein than to the subtype B protein gp140dV2.SF162. Conversely, the group immunized with the gp140dV2.SF162 immunogen showed higher antibody titers with the oligomeric subtype B protein relative its subtype C counterpart. Overall, all three assays demonstrated that high antibody cross-reactive antibodies were generated by the subtype CTV1-based DNA and protein immunogens.

The results indicate that the subtype C TV1-derived Env DNA and protein antigens are immunogenic inducing high titers of antibodies in immunized rabbits and substantial evidence of neutralizing antibodies against both subtype B and subtype C R5 virus strains. In particular, the gp140dV2.TV1 antigens have induced consistent neutralizing responses against the subtype B SF162EnvDV2 and subtype C TV2 strains. Thus, TV1-based Env DNA and protein-based antigens are immunogenic and induce high titer antibody responses reactive with both subtype C and subtype B HIV-1 Env antigens. Neutralizing antibody responses against the neutralization sensitive subtype B R5 HIV-1_{SF162DV2} strain were observed in some groups after only two DNA immunizations. Following a single booster immunization with Env protein, the majority of rabbits in groups that received V2-deleted forms of the TV1 Env showed neutralization activity against the closely related subtype C TV2 primary strain.

Example 12

Immunological Responses in Rhesus Macaques

Cellular and humoral immune responses were evaluated in three groups of rhesus macaques (each group was made up of four animals) in an immunization study structured as shown in Table I. The route of administration for the immunizing composition was electroporation in each case. Antibody titers are shown in Table I for two weeks post-second immunization.

Table I

Group	Formulation of Immunizing Composition *	Animal #	Titer
1	pCMVgag (3.5 mg) + pCMVenv (2.0 mg)	A	3,325
		B	4,000
		C (previously immunized with HCV core ISCOMS, rVVC core E1)	1,838
		D (previously immunized with HCV core ISCOMS, rVVC core E1)	1,850
2	pCMVgag (3.5 mg) + pCMVpol (4.2 mg)	A (previously immunized with HCV core ISCOMS, rVVC core E1, p55gag _{LAI} (VLP))	525
		B	5,313
		C	6,450
		D	5,713
3	pCMVgag-pol (5.0 mg)	A (previously immunized with HCV core ISCOMS, rVVC core E1, pCMVgagSF2)	0
		B (previously immunized with rVVC/E1, pCMV Epo-Epi, HIV/HCV-VLP, pCMVgagSF2, pUCgp120 SF2)	1,063
		C	513

Group	Formulation of Immunizing Composition *	Animal #	Titer
		D (previously immunized with rVVC/E1, HIV/HCV-VLP)	713

* pCMVgag = pCMVKm2.GagMod Type C Botswana
pCMVenv = pCMVLink.gp140env.dV2.TV1 (Type C)
pCMVpol = pCMVKm2.p2Pol.mut.Ina Type C Botswana
pCMVgag-pol = pCMVKm2.gagCpol.mut.Ina Type C Botswana

5

Pre-immune sera were obtained at week 0 before the first immunization. The first immunization was given at week 0. The second immunization was given at week 4. The first bleed was performed at 2 weeks post-second immunization (i.e., at week 6). A third immunization will be given at week 8 and a fourth at week 16. Animals 2A, 3A, 3B and 3D had been vaccinated previously (approximately 4 years or more) with gag plasmid DNA or gag VLP (subtype B).

Bulk CTL, ⁵¹Cr-release assays, and flow cell cytometry methods were used to obtain the data in Tables J and K. Reagents used for detecting gag- and pol-specific T-cells were (i) synthetic, overlapping peptides spanning "gagCpol" antigen (n=377), typically the peptides were pools of 15-mers with overlap by 11, the pools were as follows, pool 1, n=1-82, pool 2, n=83-164, pool 3, n=165-271, pool 4, n=272-377, accordingly pools 1 and 2 are "gag"-specific, and pools 3 and 4 are "pol"-specific, and (ii) recombinant vaccinia virus (rVV), for example, rVVgag965, rVVP2Pol975 (contains p2p7gag975), and VV_{wt}parent.

Gag-specific IFN γ + CD8 + T-cells, Gag-specific IFN γ + CD4 + T-cells, Pol-specific IFN γ + CD8 + T-cells, and Pol-specific IFN γ + CD4 + T-cells in blood were determined for each animal described in Table I above, post second immunization. The results are presented in Tables J and K. It is possible that some of the pol-specific activity shown in Table K was directed against p2p7gag.

25

Table J
Gag Assay Results

	Group/Animal	Immunizing Composition	Gag Specific CD4+ Responses				Gag Specific CD8+ Responses		
			LPA(SI)			Flow	CTL		Flow
			p55	Pool 1	Pool 2	IFNg+	Pool 1	Pool 2	IFNg+
5	1A	pCMVgag pCMVenv	3.3	5.9	3.8	496	minus	minus	225
	1B	pCMVgag pCMVenv	11.8	4.4	1.5	786	minus	minus	160
	1C	pCMVgag pCMVenv	5.7	1.1	2.4	361	plus	plus	715
	1D	pCMVgag pCMVenv	6.5	3.1	1.6	500	plus	?	596
10	2A	pCMVgag pCMVpol	4.8	4.8	1.6	405	plus	minus	1136
	2B	pCMVgag pCMVpol	12.5	6.8	3.3	1288	plus	minus	2644
	2C	pCMVgag pCMVpol	6.0	3.8	2.1	776	minus	minus	0
	2D	pCMVgag pCMVpol	18.9	13.5	5.4	1351	minus	minus	145
15	3A	pCMV gagpol	12.2	7.0	1.5	560	plus	plus	3595
	3B	pCMV gagpol	2.7	5.6	1.3	508	plus	?	3256
	3C	pCMV gagpol	11.6	5.0	1.2	289	minus	?	617
	3D	pCMV gagpol	1.5	1.2	1.4	120	minus	minus	277

? = might be positive on rVVp2Pol.

Table K
Pol Assay Results

5 Group / Animal	Immun- izing Compo- sition	Pol Specific CD4+ Response			Pol Specific CD8+ Responses		
		LPA(SI)		Flow	CTL		Flow
		Pool 3	Pool 4	IFNg+	Pool 3	Pool 4	IFNg+
1A	pCMVgag pCMVenv	1	1.2	0	minus	minus	0
1B	pCMVgag pCMVenv	1	1	0	minus	minus	0
10 1C	pCMVgag pCMVenv	1	1.1	0	minus	minus	0
1D	pCMVgag pCMVenv	1.2	1.3	0	minus	minus	262
2A	pCMVgag pCMVpol	1.1	0.9	92	minus	minus	459
2B	pCMVgag pCMVpol	2.5	1.8	107	minus	minus	838
2C	pCMVgag pCMVpol	1.2	1.1	52	plus	minus	580
15 2D	pCMVgag pCMVpol	2.5	2.7	113	plus	plus	5084
3A	pCMV gagpol	2.7	2.4	498	minus	minus	3631
3B	pCMV gagpol	1.1	1	299	minus	minus	1346
3C	pCMV gagpol	2.1	1.4	369	minus	minus	399
20 3D	pCMV gagpol	1.3	1.8	75	minus	minus	510

These results support that the constructs of the present invention are capable of generating specific cellular and humoral responses against the selected HIV-polypeptide antigens.

Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

What is claimed is:

1. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:19.
2. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to at least 500 contiguous nucleotides of SEQ ID NO:12 or SEQ ID NO:20.
3. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29 and SEQ ID NO:30.
4. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, and SEQ ID NO:38.
5. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Int* polypeptide, wherein the polynucleotide sequence encoding said *Int* polypeptide comprises a sequence having at least 95% sequence identity to SEQ ID NO:39.

6. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Int* polypeptide, wherein the polynucleotide sequence encoding said *Int* polypeptide comprises a sequence having at least 98% sequence identity to SEQ ID NO:40.
- 5
7. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Nef* polypeptide, wherein the polynucleotide sequence encoding said *Nef* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:41 or SEQ ID NO:203.
- 10
8. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *p15RNaseH* polypeptide, wherein the polynucleotide sequence encoding said *p15RNaseH* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:42.
- 15
9. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Pol* polypeptide, wherein the polynucleotide sequence encoding said *Pol* polypeptide comprises a sequence having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.
- 20
10. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Tat* polypeptide, wherein the polynucleotide sequence encoding said *Tat* polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO:46, SEQ ID NO:47 and SEQ ID NO:48.
- 25
11. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Prot* polypeptide, wherein the polynucleotide sequence encoding said *Prot* polypeptide comprises a sequence having at least 95% sequence identity to SEQ ID NO:49 or SEQ ID NO:50.
- 30

12. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Prot* polypeptide, wherein the polynucleotide sequence encoding said *Prot* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:51.
- 5
13. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Rev* polypeptide, wherein the polynucleotide sequence encoding said *Rev* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:52.
- 10
14. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Tat* polypeptide, wherein the polynucleotide sequence encoding said *Tat* polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:60.
- 15
15. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190 and SEQ ID NO:191.
- 20
16. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of any of claims 1 to 15, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.
- 25
17. The recombinant expression system of claim 16, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription
- 30

enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

- 5 18. The recombinant expression system of claim 16, wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.
- 10 19. A cell comprising an expression cassette of any of claims 1 to 15, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.
- 20 20. The cell of claim 19, wherein the cell is a mammalian cell.
- 15 21. The cell of claim 20, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.
- 20 22. The cell of claim 21, wherein said cell is a CHO cell.
- 20 23. The cell of claim 19, wherein the cell is an insect cell.
24. The cell of claim 23, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.
- 25 25. The cell of claim 19, wherein the cell is a bacterial cell.
26. The cell of claim 19, wherein the cell is a yeast cell.
27. The cell of claim 19, wherein the cell is a plant cell.
- 30 28. The cell of claim 19, wherein the cell is an antigen presenting cell.

29. The cell of claim 28, wherein the antigen presenting cell is a lymphoid cell selected from the group consisting of macrophages, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.
- 5 30. The cell of claim 19, wherein the cell is a primary cell.
31. The cell of claim 19, wherein the cell is an immortalized cell.
32. The cell of claim 19, wherein the cell is a tumor-derived cell.
- 10 33. A method for producing a polypeptide including HIV *Gag* polypeptide sequences, said method comprising,
 incubating the cells of claim 19, under conditions for producing said polypeptide.
- 15 34. A gene delivery vector for use in a mammalian subject, comprising
 a suitable gene delivery vector for use in said subject, wherein the vector comprises an expression cassette any of claims 1 to 15, and wherein said polynucleotide sequence is operably linked to control elements compatible with
20 expression in the subject.
35. A method of DNA immunization of a subject, comprising,
 introducing a gene delivery vector of claim 34 into said subject under conditions that are compatible with expression of said expression cassette in said
25 subject.
36. The method of claim 35, wherein said gene delivery vector is a nonviral vector.
37. The method of claim 35, wherein said vector is delivered using a particulate
30 carrier.

38. The method of claim 37, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said subject using a gene gun.
39. The method of claim 35, wherein said vector is encapsulated in a liposome preparation.
40. The method of claim 35, wherein said vector is a viral vector.
41. The method of claim 40, wherein said viral vector is a retroviral vector.
42. The method of claim 40, wherein said viral vector is an alphaviral vector.
43. The method of claim 40, wherein said viral vector is a lentiviral vector.
44. The method of claim 35, wherein said subject is a mammal.
45. The method of claim 44, wherein said mammal is a human.
46. A method of generating an immune response in a subject, comprising transfecting cells of said subject a gene delivery vector of claim 34, under conditions that permit the expression of said polynucleotide and production of said polypeptide, thereby eliciting an immunological response to said polypeptide.
47. The method of claim 46, wherein said vector is a nonviral vector.
48. The method of claim 46, wherein said vector is delivered using a particulate carrier.
49. The method of claim 46, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said vertebrate cell using a gene gun.

50. The method of claim 46, wherein said vector is encapsulated in a liposome preparation.
51. The method of claim 46, wherein said vector is a viral vector.
52. The method of claim 51, wherein said viral vector is a retroviral vector.
53. The method of claim 51, wherein said viral vector is an alphaviral vector.
54. The method of claim 51, wherein said viral vector is a lentiviral vector.
55. The method of claim 46, wherein said subject is a mammal.
56. The method of claim 55, wherein said mammal is a human.
57. The method of claim 46, wherein said transfecting is done *ex vivo* and said transfected cells are reintroduced into said subject.
58. The method of claim 46, wherein said transfecting is done *in vivo* in said subject.
59. The method of claim 46, where said immune response is a humoral immune response.
60. The method of claim 46, where said immune response is a cellular immune response.
61. The method of claim 46, wherein the gene delivery vector is administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermally, intravaginally, intrarectally, orally or intravenously.

8_5_ZA

1 TGGAAGGGTT AATTTACTCC AAGAAAAGGC AAGAAATCCT TGATTTGTGG GTCTATCACA
 61 CACAAGGCTT CTTCCCTGAT TGGCAAAACT ACACACCGGG GCCAGGGGTC AGATATCCAC
 121 TGACCTTTGG ATGGTGCTAC AAGCTAGTGC CAGTTGACCC AGGGGAGGTG GAAGAGGCCA
 181 ACGGAGGAGA AGACAACGTG TTGCTACACC CTATGAGCCA ACATGGAGCA GAGGATGAAG
 241 ATAGAGAAGT ATTAAAGTGG AAGTTTGACA GCCTCCTAGC ACGCAGACAC ATGGCCCGCG
 301 AGCTACATCC GGAGTATTAC AAAGACTGCT GACACAGAAG GGACTTTCCG CCTGGGACTT
 361 TCCACTGGGG CGTTCCGGGA GGTGTGGTCT GGGCGGGACT TGGGAGTGGT CAACCCTCAG
 421 ATGCTGCATA TAAGCAGCTG CTTTTGCGCT GTACTGGGTC TCTCTCGGTA GACCAGATCT
 481 GAGCCTGGGA GCCCTCTGGC TATCTAGGGA ACCCACTGCT TAAGCCTCAA TAAAGCTTGC
 541 CTTGAGTGTCT TTAAGTAGTG TGTGCCATC TGTGTGTGA CTCTGGTAAC TAGAGATCCC
 601 TCAGACCCTT TGTGGTAGTG TGGAAAATCT CTAGCAGTGG CGCCGAACA GGGACCAGAA
 661 AGTGAAAGTG AGACCAGAGG AGATCTCTCG ACGCAGGACT CGGCTTGCTG AAGTGCACAC
 721 GGCAAGAGGC GAGAGGGGCG GCTGGTGAGT ACGCCAATTT TACTTGACTA GCGGAGGCTA
 781 GAAGGAGAGA GATGGGTGCG AGAGCGTCAA TATTAAGCGG CGGAAAATTA GATAAATGGG
 841 AAAGAATTAG GTTAAGGCCA GGGGAAAGA AACATTATAT GTTAAACAT CTAGTATGGG
 901 CAAGCAGGGA GCTGGAAAGA TTGCACTTA ACCCTGGCCT GTTAGAAACA TCAGAAGGCT
 961 GTAAACAAAT AATAAACAG CTACAACCAG CTCTTCAGAC AGGAACAGAG GAACTTAGAT
 1021 CATTATTCAA CACAGTAGCA ACTCTCTATT GTGTACATAA AGGGATAGAG GTACGAGACA
 1081 CCAAGGAAGC CTTAGACAAG ATAGAGGAAG AACAAAACAA ATGTCAGCAA AAAGCACAAC
 1141 AGGCAAAAGC AGCTGACGAA AAGGTCAGTC AAAATTATCC TATAGTACAG AATGCCCAAG
 1201 GGCAAAATGGT ACACCAAGCT ATATCACCTA GAACATTGAA TGCATGGATA AAAGTAATAG
 1261 AGGAAAAGGC TTTCAATCCA GAGGAAATAC CCATGTTTAC AGCATTATCA GAAGGAGCCA
 1321 CCCACAAGA TTTAAACACA ATGTTAAATA CAGTGGGGGG ACATCAAGCA GCCATGCAAA
 1381 TGTAAAAAGA TACCATCAAT GAGGAGGCTG CAGAATGGGA TAGGACACAT CCAGTACATG
 1441 CAGGGCCTGT TGCACCAGC CAGATGAGAG AACCAAGGGG AAGTGACATA GCAGGAACATA
 1501 CTAGTACCCT TCAGGAACAA ATAGCATGGA TGACAAGTAA TCCACCTATT CCAGTAGAAG
 1561 ACATCTATAA AAGATGGATA ATTCTGGGGT TAAATAAAAT AGTAAGAATG TATAGCCCTG
 1621 TTAGCATTTT GGACATAAAA CAAGGGCCAA AAGAACCCTT TAGAGACTAT GTAGACCGGT
 1681 TCTTTAAAC CTTAAGAGCT GAACAAGCTA CACAAGATGT AAAGAATTGG ATGACAGACA
 1741 CCTTGTGGT CCAAAATGCG AACCCAGATT GTAAGACCAT TTAAAGAGCA TTAGGACCAG
 1801 GGGCCTCATT AGAAGAAATG ATGACAGCAT GTCAGGGAGT GGGAGGACCT AGCCATAAAG
 1861 CAAGAGTGTG GGCAGAGGCA ATGAGCCAAG CAAACAGTAA CATACTAGTG CAGAGAAGCA
 1921 ATTTTAAAGG CTCTAACAGA ATTATTAAAT GTTTCAACTG TGGCAAAGTA GGGCACATAG
 1981 CCAGAAATTG CAGGGCCCCT AGGAAAAGG GCTGTTGGAA ATGTGGACAG GAAGGACACC
 2041 AAATGAAAGA CTGTACTGAG AGGCAGGCTA ATTTTTTAGG GAAAATTGG CCTTCCACA
 2101 AGGGGAGGCC AGGGAATTTT CTCCAGAACA GACCAGAGCC AACAGCCCCA CCAGCAGAAC
 2161 CAACAGCCCC ACCAGCAGAG AGCTTCAGGT TCGAGGAGAC AACCCTCGTG CCGAGGAAGG
 2221 AGAAAGAGAG GGAACCTTTA ACTTCCCTCA AATCACTCTT TGGCAGCGAC CCCTTGTCTC
 2281 AATAAAAGTA GAGGGCCAGA TAAAGGAGGC TCTCTTAGAC ACAGGAGCAG ATGATACAGT
 2341 ATTAGAAGAA ATAGATTGCG CAGGGAAATG GAAACCAAAA ATGATAGGGG GAATTGGAGG
 2401 TTTTATCAAA GTAAGACAGT ATGATCAAAT ACTTATAGAA ATTTGTGGAA AAAAGGCTAT
 2461 AGGTACAGTA TTAGTAGGGC CTACACCAGT CAACATAATT GGAAGAAATC TGTTAACTCA
 2521 GCTTGGATGC ACCTAAATT TTCCAATTAG TCCTATTGAA ACTGTACCAG TAAATTTAA
 2581 ACCAGGAATG GATGGCCCAA AGGTCAAACA ATGGCCATTG ACAGAAGAAA AATAAAAGC
 2641 ATTAACAGCA ATTTGTGAGG AAATGGAGAA GGAAGGAAA ATTACAAAAA TTGGGCTGA
 2701 TAATCCATAT AACACTCCAG TATTTGCCAT AAAAAAGAAG GACAGTACTA AGTGGAGAAA
 2761 ATTAGTAGAT TTCAGGGAAC TCAATAAAG AACTCAAGAC TTTTGGGAAG TTCAATTAGG
 2821 AATACCACAC CCAGCAGGAT TAAAAAAGAA AAAATCAGTG ACAGTGCTAG ATGTGGGGGA
 2881 TGCATATTTT TCAGTTCCTT TAGATGAAG CTTGAGGAAA TATACTGCAT TCACCATACC

FIGURE 1A

2941 TAGTATAAAC AATGAAACAC CAGGGATTAG ATATCAATAT AATGTGCTGC CACAGGGATG
 3001 GAAAGGATCA CCAGCAATAT TCCAGAGTAG CATGACAAAA ATCTTAGAGC CCTTCAGAGC
 3061 AAAAAATCCA GACATAGTTA TCTATCAATA TATGGATGAC TTGTATGTAG GATCTGACTT
 3121 AGAAATAGGG CAACATAGAG CAAAAATAGA AGAGTTAAGG GAACATTTAT TGAATGGGG
 3181 ATTTACAACA CCAGACAAGA AACATCAAAA AGAACCCCCA TTTCTTTGGA TGGGGTATGA
 3241 ACTCCATCCT GACAAATGGA CAGTACAACC TATACTGCTG CCAGAAAAGG ATAGTTGGAC
 3301 TGTCAATGAT ATACAGAAGT TAGTGGGAAA ATTAACTGG GCAAGTCAGA TTTACCCAGG
 3361 GATTAAAGTA AGGCAACTCT GTAAACTCCT CAGGGGGGCC AAAGCACTAA CAGACATAGT
 3421 ACCACTAACT GAAGAAGCAG AATTAGAATT GGCAGAGAAC AGGGAAATTT TAAGAGAACC
 3481 AGTACATGGA GTATATTATG ATCCATCAAA AGACTTGATA GCTGAAATAC AGAAACAGGG
 3541 GCATGAACAA TGGACATATC AAATTTATCA AGAACCATTT AAAAATCTGA AAACAGGGAA
 3601 GTATGCAAAA ATGAGGACTA CCCACACTAA TGAATGTAATA CAGTTAACAG AGGCAGTGCA
 3661 AAAAAATAGCC ATGGAAAGCA TAGTAATATG GGGAAAGACT CCTAAATTTA GACTACCCAT
 3721 CCAAAAAGAA ACATGGGAGA CATGGTGGAC AGACTATTGG CAAGCCACCT GGATCCCTGA
 3781 GTGGGAGTTT GTTAATACCC CTCCCCTAGT AAAATTATGG TACCAACTAG AAAAGATCC
 3841 CATAGCAGGA GTAGAACTT TCTATGTAGA TGGAGCAACT AATAGGGAAG CTAAATAGG
 3901 AAAAGCAGGG TATGTTACTG ACAGAGGAAG GCAGAAAATT GTTACTCTAA CTAACACAAC
 3961 AAATCAGAAG ACTGAGTTAC AAGCAATTCA GCTAGCTCTG CAGGATTCAG GATCAGAAGT
 4021 AAACATAGTA ACAGACTCAC AGTATGCATT AGGAATCATT CAAGCACAAC CAGATAAGAG
 4081 TGACTCAGAG ATATTAAACC AAATAATAGA ACAGTTAATA AACAAGGAAA GAATCTACCT
 4141 GTCATGGGTA CCAGCACATA AAGGAATTGG GGGAAATGAA CAAGTAGATA AATTAGTAAG
 4201 TAAGGGAAAT AGGAAAGTGT TGTTCCTAGA TGGAAATAGAT AAAGCTCAAG AAGAGCATGA
 4261 AAGGTACCAC AGCAATTGGA GAGCAATGGC TAATGAGTTT AATCTGCCAC CCATAGTAGC
 4321 AAAAGAAATA GTAGCTAGCT GTGATAAATG TCAGCTAAAA GGGGAAGCCA TACATGGACA
 4381 AGTCGACTGT AGTCCAGGGA TATGGCAATT AGATTGTACC CATTTAGAGG GAAAAATCAT
 4441 CCTGGTAGCA GTCCATGTAG CTAGTGGCTA CATGGAAGCA GAGGTTATCC CAGCAGAAAC
 4501 AGGACAAGAA ACAGCATATT TTATATTAAA ATTAGCAGGA AGATGGCCAG TCAAAGTAAT
 4561 ACATACAGAC AATGGCAGTA ATTTTACCAG TACTGCAGTT AAGGCAGCCT GTTGGTGGGC
 4621 AGGTATCCAA CAGGAATTTG GAATTCCTTA CAATCCCCAA AGTCAGGGAG TGGTAGAATC
 4681 CATGAATAAA GAATTAAAGA AAATAATAGG ACAAGTAAGA GATCAAGCTG AGCACCTTAA
 4741 GACAGCAGTA CAAATGGCAG TATTCATTCA CAATTTTAAA AGAAAAGGGG GAATTGGGGG
 4801 GTACAGTGCA GGGGAAGAA TAATAGACAT AATAGCAACA GACATACAAA CTAAGAATT
 4861 AAAAAACAA ATTATAAGAA TTCAAATTT TOGGGTTTAT TACAGAGACA GCAGAGACCC
 4921 TATTTGGAAA GGACCAGCCG AACTACTCTG GAAAGGTGAA GGGGTAGTAG TAATAGAAGA
 4981 TAAAGGTGAC ATAAAGGTAG TACCAAGGAG GAAAGCAAAA ATCATTAGAG ATTATGGAAA
 5041 ACAGATGGCA GGTGCTGATT GTGTGGCAGG TGGACAGGAT GAAGATTAGA GCATGGAATA
 5101 GTTTAGTAAA GCACCATATG TATATATCAA GGAGAGCTAG TGGATGGGTC TACAGACATC
 5161 ATTTTGAAAG CAGACATCCA AAAGTAAGTT CAGAAGTACA TATCCCATTA GGGGATGCTA
 5221 GATTAGTAAT AAAAACATAT TGGGGTTTGC AGACAGGAGA AAGAGATTGG CATTTGGGTC
 5281 ATGGAGTCTC CATAGAATGG AGACTGAGAG AATACAGCAC ACAAGTAGAC CCTGACCTGG
 5341 CAGACCAGCT AATTCACATG CATTATTTT ATTGTTTTAC AGAATCTGCC ATAAGACAAG
 5401 CCATATTAGG ACACATAGTT TTTCCCTAGGT GTGACTATCA AGCAGGACAT AAGAAGGTAG
 5461 GATCTCTGCA ATACTTGGCA CTGACAGCAT TGATAAAACC AAAAAAGAGA AAGCCACCTC
 5521 TGCCTAGTGT TAGAAAATTA GTAGAGGATA GATGGAAACG CCCCAGAG ACCAGGGGCC
 5581 GCAGAGGGAA CCATACAATG AATGGACACT AGAGATTCTA GAAGAACTCA AGCAGGAAGC
 5641 TGTCAGACAC TTTCTAGAC CATGGCTCCA TAGCTTAGGA CAATATATCT ATGAAACCTA
 5701 TGGGGATACT TGGACGGGAG TTGAAGCTAT AATAAGAGTA CTGCAACAAC TACTGTTTAT
 5761 TCATTTTCTA ATTGGATGCC AACATAGCAG AATAGGCATC TTGCGACAGA GAAGAGCAAG
 5821 AAATGGAGCC AGTAGATCCT AAATAAAGC CCTGGAACCA TCCAGGAAGC CAACCTAAAA
 5881 CAGCTTGTA TAATTGCTTT TGCAAACT GTAGCTATCA TTGTCTAGTT TGCTTTCAGA

FIGURE 1B

5941 CAAAAGGTTT AGGCATTTC TATGGCAGGA AGAAGCGGAG ACAGCGACGA AGCGCTCCTC
 6001 CAAGTGGTGA AGATCATCAA AATCCTCTAT CAAAGCAGTA AGTACACATA GTAGATGTAA
 6061 TGGTAAGTTT AAGTTTATTT AAAGGAGTAG ATTATAGATT AGGAGTAGGA GCATTGATAG
 6121 TAGCACTAAT CATAGCAATA ATAGTGTGGA CCATAGCATA TATAGAATAT AGGAAATTGG
 6181 TAAGACAAAA GAAAATAGAC TGGTTAATTA AAAGAATTAG GGAAAGAGCA GAAGACAGTG
 6241 GCAATGAGAG TGATGGGGAC ACAGAAGAAT TGTCAACAAT GGTGGATATG GGGCATCTTA
 6301 GGCTTCTGGA TGCTAATGAT TTGTAACACG GAGGACTTGT GGGTCACAGT CTACTATGGG
 6361 GTACCTGTGT GGAGAGAAGC AAAAATACT CTATTCTGTG CATCAGATGC TAAAGCATAT
 6421 GAGACAGAAG TGCATAATGT CTGGGCTACA CATGCTTGTG TACCCACAGA CCCCACCCCA
 6481 CAAGAAATAG TTTTGGGAAA TGTAACAGAA AATTTTAAATA TGTGGAAAAA TAACATGGCA
 6541 GATCAGATGC ATGAGGATAT AATCAGTTTA TGGGATCAAA GCCTAAAGCC :ATGTGTAAAG
 6601 TTGACCCAC TCTGTGTCAC TTTAACTGT ACAGATACAA ATGTTACAGG TAATAGAACT
 6661 GTTACAGGTA ATACAAATGA TACCAATATT GCAAATGCTA CATATAAGTA TGAAGAAATG
 6721 AAAAATTGCT CTTTCAATGC AACCACAGAA TTAAGAGATA AGAAACATAA AGAGTATGCA
 6781 CTCTTTTATA AACTGATAT AGTACCCTT AATGAAAATA GTAACAATT TACATATAGA
 6841 TTAATAAATT GCAATACCTC AACCATAACA CAAGCCTGTC CAAAGGTCTC TTTTGACCCG
 6901 ATTCTTATAC ATTACTGTGC TCCAGCTGAT TATGCGATTG TAAAGTGTA TAATAAGACA
 6961 TTCAATGGGA CAGGACCATG TTATAATGTC AGCACAGTAC AATGTACACA TGGAAATTAAG
 7021 CCAGTGGTAT CAACTCAACT ACTGTTAAAT GGTAGTCTAG CAGAAGAAGG GATAATAATT
 7081 AGATCTGAAA ATTTGACAGA GAATACCAAA ACAATAATAG TACATCTTAA TGAATCTGTA
 7141 GAGATTAATT GTACAAGGCC CAACAATAAT ACAAGGAAAA GTGTAAGGAT AGGACCAGGA
 7201 CAAGCATTCT ATGCAACAAA TGACGTAATA GGAACATAA GACAAGCACA TTGTAACATT
 7261 AGTACAGATA GATGGAATAA AACTTTACAA CAGGTAATGA AAAAATTAGG AGAGCATTTC
 7321 CCTAATAAAA CAATAAAATT TGAACCATAT GCAGGAGGGG ATCTAGAAAT TACAATGCAT
 7381 AGCTTTAATT GTAGAGGAGA ATTTTCTAT TGCAATACAT CAAACCTGTT TAATAGTACA
 7441 TACTACCCTA AGAATGGTAC ATACAAATAC AATGGTAATT CAAGCTTACC CATCACACTC
 7501 CAATGCAAAA TAAAACAAAT TGTACGCATG TGGCAAGGGG TAGGACAAGC AATGTATGCC
 7561 CCTCCCATG CAGGAAACAT AACATGTAGA TCAAACATCA CAGGAATACT ATTGACACGT
 7621 GATGGGGGAT TTAACAACAC AAACAACGAC ACAGAGGAGA CATTAGAGC TGGAGGAGGA
 7681 GATATGAGGG ATAACGGAG AAGTGAATTA TATAAATATA AAGTGGTAGA AATTAAGCCA
 7741 TTGGGAATAG CACCCACTAA GGCAAAAGA AGAGTGGTGC AGAGAAAAAA AAGAGCAGTG
 7801 GGAATAGGAG CTGTGTTCTT TGGGTTCTTG GGAGCAGCAG GAAGCACTAT GGGCGCAGCG
 7861 TCAATAACGC TGACGGTACA GGCCAGACAA CTGTTGTCTG GTATAGTGCA ACAGCAAAGC
 7921 AATTTGCTGA AGGCTATAGA GGCGCAACAG CATATGTTGC AACTCACAGT CTGGGGCATT
 7981 AAGCAGCTCC AGGCGAGAGT CCTGGCTATA GAAAGATACC TAAAGGATCA ACAGCTCCTA
 8041 GGGATTGTTGG GCTGCTCTGG AAGACTCATC TGCAACACTG CTGTGCCTTG GAACTCCAGT
 8101 TGGAGTAATA AATCTGAAGC AGATATTTGG GATAACATGA CTGGGATGCA GTGGGATAGA
 8161 GAAATTAAATA ATTACACAGA AACAAATATC AGGTTGCTTG AAGACTGCA AAACCAGCAG
 8221 GAAAAGAATG AAAAAGATTT ATTAGAATTG GACAAGTGGA ATAATCTGTG GAATTGGTTT
 8281 GACATATCAA ACTGGCTGTG GTATATAAAA ATATTCTATA TGATAGTAGG AGGCTTGATA
 8341 GGTTTAAGAA TAATTTTTCG TGTGCTCTCT ATAGTGAATA GAGTTAGGCA GGGATACTCA
 8401 CCTTTGTCAT TTCAGACCCT TACCCCAAGC CCGAGGGGAC TCGACAGGCT CGGAGGAATC
 8461 GAAGAAGAAG GTGGAGAGCA AGACAGAGAC AGATCCATAC GATTGGTGAG CGGATTCTTG
 8521 TCGCTTGCCT GGGACGATCT GCGAGCCCTG TGCCCTTCA GCTACCACCG CTTGAGAGAC
 8581 TTCATATTAA TTGCAGTGAG GGCAGTGGA CTTCTGGGAC ACAGCAGTCT CAGGGGACTA
 8641 CAGAGGGGGT GGGAGATCCT TAAGTATCTG GGAAGTCTTG TGCAGTATTG GGGTCTAGAG
 8701 CTAAAAAGA GTGCTATTAG TCCGCTTGAT ACCATAGCAA TAGCAGTAGC TGAAGGAACA
 8761 GATAGGATTA TAGAATTGGT ACAAAGAATT TGTAGAGCTA TCCTCAACAT ACCTAGGAGA
 8821 ATAAGACAGG GCTTTGAAGC AGCTTTGCTA TAAATGGGA GGCAAGTGGT CAAACGCAG
 8881 CATAGTTGGA TGGCCTGCAG TAAGAGAAAG AATGAGAAGA ACTGAGCCAG CAGCAGAGGG
 8941 AGTAGGAGCA GGTCTCAAG ACTTAGATAG ACATGGGGCA CTTACAAGCA GCAACACACC

FIGURE 1C

9001 TGCTACTAAT GAAGCTTGTG CCTGGCTGCA AGCACAAGAG GAGGACGGAG ATGTAGGCTT
9061 TCCAGTCAGA CCTCAGGTAC CTTTAAGACC AATGACTTAT AAGAGTGCAG TAGATCTCAG
9121 CTTCTTTTFA AAAGAAAAGG GGGGACTGGA AGGGTTAATT TACTCTAGGA AAAGGCAAGA
9181 AATCCTTGAT TTGTGGGTCT ATAACACACA AGGCTTCTTC CCTGATTGGC AAAACTACAC
9241 ATCGGGGCCA GGGGTCCGAT TCCCACTGAC CTTTGGATGG TGCTTCAAGC TAGTACCAGT
9301 TGACCCAAGG GAGGTGAAAG AGGCCAATGA AGGAGAAGAC AACTGTTTGC TACACCCTAT
9361 GAGCCAACAT GGAGCAGAGG ATGAAGATAG AGAAGTATTA AAGTGGAAGT TTGACAGCCT
9421 TCTAGCACAC AGACACATGG CCCGCGAGCT ACATCCGGAG TATTACAAAG ACTGCTGACA
9481 CAGAAGGGAC TTTCCGCCTG GGACTTTCCA CTGGGGCGTT CCGGGAGGTG TGGTCTGGGC
9541 GGGACTTGGG AGTGGTCACC CTCAGATGCT GCATATAAGC AGCTGCTTTT CGCTTGACT
9601 GGGTCTCTCT CGGTAGACCA GATCTGAGCC TGGGAGCTCT CTGGCTATCT AGGGAACCCA
9661 CTGCTTAGGC CTCAATAAAG CTTGCCTTGA GTGCTCTAAG TAGTGTGTGC CCATCTGTTG
9721 TGTGACTCTG GTAAC TAGAG ATCCCTCAGA CCCTTTGTGG TAGTGTGGAA AATCTCTAGC
9781 A

FIGURE 1D

↓: is the regions for β -sheet deletions

*: is the N-linked glycosylation sites for subtype C TV1 and TV2. Possible mutation (N \rightarrow Q) or deletions can be performed.

		1		50
SF162	(1)	----	MDAMKRGGLCCMLLCCGAVFVSPSAVEKLLWVTVVYGVPVWKEATHITL	
TV1.8_2	(1)		MRVMGTQKNCQQWWIWGILGFWMLMICNTEDLWVTVVYGVPVWRDAKHTTL	
TV1.8_5	(1)		MRVMGTQKNCQQWWIWGILGFWMLMICNTEDLWVTVVYGVPVWRDAKHTTL	
TV2.12-5/1	(1)		MRARGILKNYRHWWIWGILGFWMLMICNVKGLWVTVVYGVPVGRKAKHTTL	
Consensus	(1)		MRVMGTQKNCQQWWIWGILGFWMLMICNVEDLWVTVVYGVPVWREAKHTTL	
		51		100
SF162	(47)		FCASDAKAYDTEVHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNMVD	
TV1.8_2	(51)		FCASDAKAYETEVEHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNMVD	
TV1.8_5	(51)		FCASDAKAYETEVEHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNMVD	
TV2.12-5/1	(51)		FCASDAKAYEKEVEHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNMVD	
Consensus	(51)		FCASDAKAYETEVEHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNMVD	
			β 2/V1V2/ β 3	
		101		150
SF162	(97)		QMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVTGNRTVTGNSNSN	
TV1.8_2	(101)		QMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVTGNRTVTGNSNSN	
TV1.8_5	(101)		QMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVTGNRTVTGNSNSN	
TV2.12-5/1	(101)		QMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVTGNRTVTGNSNSN	
Consensus	(101)		QMHEDIISLWDQSLKPCVKLTPLCVTLNCTNVTGNRTVTGNSNSN	
		151		200
SF162	(139)		WKEMDRGELENGSFKNVHSEINMOKKEYALFYKLDIVPTDN	
TV1.8_2	(151)		TCIYNIEEMKNCSENAITELDKKHKEYALFYKLDIVPLN	
TV1.8_5	(151)		NATYKYEEMKNCSENAITELDKKHKEYALFYKLDIVPLN	
TV2.12-5/1	(141)		-----KMKNCSEYVITELDKKHKEYALFYKLDIVPLN	
Consensus	(151)		A Y EEMKNCSEFNVITELDKKHKEYALFYKLDIVPLN	
		201		250
SF162	(185)		RLINCNTSTITQACPKVSEFPIPIHYCAPAGYAILKCNKTFNGTGPCYN	
TV1.8_2	(199)		RLINCNTSTITQACPKVSEFPIPIHYCAPAGYAILKCNKTFNGTGPCYN	
TV1.8_5	(199)		RLINCNTSTITQACPKVSEFPIPIHYCAPAGYAILKCNKTFNGTGPCYN	
TV2.12-5/1	(185)		RLINCNTSALTQACPKVSEFPIPIHYCAPAGYAILKCNKTFNGTGPCYN	
Consensus	(201)		RLINCNTSTITQACPKVSEFPIPIHYCAPAGYAILKCNKTFNGTGPCYN	
		251		300
SF162	(235)		VSTVQCTHGIRPVVSTOLLNGSLAEEGVIRSENLTENTKTIIVHLNES	
TV1.8_2	(249)		VSTVQCTHGIRPVVSTOLLNGSLAEEGVIRSENLTENTKTIIVHLNES	
TV1.8_5	(249)		VSTVQCTHGIRPVVSTOLLNGSLAEEGVIRSENLTENTKTIIVHLNES	
TV2.12-5/1	(235)		VSTVQCTHGIRPVVSTOLLNGSLAEEGVIRSENLTENTKTIIVHLNES	
Consensus	(251)		VSTVQCTHGIRPVVSTOLLNGSLAEEGVIRSENLTENTKTIIVHLNES	
		301		350
SF162	(285)		VEINCTRPNNNTRKSGITIGPGRATYATNDVIGNTROAHCNISTDRWNKTL	
TV1.8_2	(299)		VEINCTRPNNNTRKSVRIGPQAFYATNDVIGNTROAHCNISTDRWNKTL	
TV1.8_5	(299)		VEINCTRPNNNTRKSVRIGPQAFYATNDVIGNTROAHCNISTDRWNKTL	
TV2.12-5/1	(285)		VEINCTRPNNNTRKSVRIGPQAFYATNDVIGNTROAHCNISTDRWNKTL	
Consensus	(301)		VEINCTRPNNNTRKSVRIGPQAFYATNDVIGNTROAHCNISTDRWNKTL	

FIGURE 2A

		351	*		*	400
SF162	(335)	KQIVTKLQAOFGNKT	-	IVFKQSGGDPPEIVMHSFNCGGEEFYCNSTQIFN		
TV1.8_2	(349)	QQVMKKGGEHFPNKT	-	LQFKPHAGGDLEITMHSFNCRGEFFYCNTSNLFN		
TV1.8_5	(349)	QQVMKKGGEHFPNKT	-	IKKPEPHAGGDLEITMHSFNCRGEFFYCNTSNLFN		
TV2.12-5/1	(335)	QRVSOKLQELFPNST	-	GIKKAPHBGGDLEITTHSTNCGGEEFYCNTHIDIFN		
Consensus	(351)	QQVMKKLQEHFPNKT	-	IKFKPHAGGDLEITMHSFNCRGEFFYCNTSNLFN		
		401	*		*	450
SF162	(384)	STWNN-----TIGPN-NINGTI	TLP	GRFKQLINRQGVQKAMYAPPIRG		
TV1.8_2	(398)	STVHS---NNGTYKINGNSSSP	ITLQCKIKQIIRMWQGVQAMYPPIAG			
TV1.8_5	(398)	STVMP---KNGTYKINGNSSLP	ITLQCKIKQIIRMWQGVQAMYPPIAG			
TV2.12-5/1	(385)	STYSNGTCTNGTCSN--NTERIT	LOCRKQLINRQGVQKAMYAPPIAG			
Consensus	(401)	STYHN	NGTYKINGNSS	PITLQCKIKQIIRMWQGVQAMYPPIAG		
		451	*		*	500
SF162	(427)	QITRGSNITGLILLTRDGGKEISNT	--	TETFRPGGGMDRDNWRSELYKYKV		
TV1.8_2	(445)	NITCRSNITGLILLTRDGGFNNTNN	--	TETFRPGGGMDRDNWRSELYKYKV		
TV1.8_5	(445)	NITCRSNITGLILLTRDGGFNNTNND	TETFRPGGGMDRDNWRSELYKYKV			
TV2.12-5/1	(433)	NITCRSNITGLILLTRDGGDNNTET	--	TETFRPGGGMDRDNWRSELYKYKV		
Consensus	(451)	NITCRSNITGILLTRDGGFNNTNT		TETFRPGGGMDRDNWRSELYKYKV		
		501				550
SF162	(475)	VKTEPLGVAPTKAKRRVVOREKRAVIT	GAMTGHLCAGSTMGASITILT			
TV1.8_2	(493)	VEIKPLGIAPTKAKRRVVOREKRAVGIG	AVFLGFLGAGSTMGASITILT			
TV1.8_5	(495)	VEIKPLGIAPTKAKRRVVOREKRAVGIG	AVFLGFLGAGSTMGASITILT			
TV2.12-5/1	(480)	VEIKPLGVAPTKAKRRVVOREKRAVGIG	AVFLGFLGAGSTMGASITILT			
Consensus	(501)	VEIKPLGIAPTKAKRRVVOREKRAVGIG	AVFLGFLGAGSTMGASITILT			
		551				600
SF162	(525)	VQARQLLSGLVQQGSNLLKATEAQOH	MLQLTWVGIKQLQARVLATERYLK			
TV1.8_2	(543)	VQARQLLSGLVQQGSNLLKATEAQOH	MLQLTWVGIKQLQARVLATERYLK			
TV1.8_5	(545)	VQARQLLSGLVQQGSNLLKATEAQOH	MLQLTWVGIKQLQARVLATERYLK			
TV2.12-5/1	(530)	VQARQLLSGLVQQGSNLLKATEAQOH	MLQLTWVGIKQLQARVLATERYLK			
Consensus	(551)	VQARQLLSGLVQQGSNLLKATEAQOH	MLQLTWVGIKQLQARVLATERYLK			
		601	*	*	*	650
SF162	(575)	DQQLLGWGCSEKLICTTAVPWNSSWSN	KSEADWDNMWQWDREISNY			
TV1.8_2	(593)	DQQLLGWGCSEKLICTTAVPWNSSWSN	KSEADWDNMWQWDREISNY			
TV1.8_5	(595)	DQQLLGWGCSEKLICTTAVPWNSSWSN	KSEADWDNMWQWDREISNY			
TV2.12-5/1	(580)	DQQLLGWGCSEKLICTTAVPWNSSWSN	KSEADWDNMWQWDREISNY			
Consensus	(601)	DQQLLGWGCSEKLICTTAVPWNSSWSN	KSEADWDNMWQWDREISNY			
		651				700
SF162	(625)	TNLYTYTLEESONQOEKNEKDILLED	DKWNNLWNNWFDISNWLWYIKIFIMI			
TV1.8_2	(643)	TGLTYNILEDSONQOEKNEKDILLED	DKWNNLWNNWFDISNWLWYIKIFIMI			
TV1.8_5	(645)	TETTYRILEDSONQOEKNEKDILLED	DKWNNLWNNWFDISNWLWYIKIFIMI			
TV2.12-5/1	(630)	TNTTYRILEDSQSQOEKNEKDILLED	DKWNNLWNNWFDISNWLWYIKIFIMI			
Consensus	(651)	TNTTYRILEDSQSQOEKNEKDILLED	DKWNNLWNNWFDISNWLWYIKIFIMI			
		701				750
SF162	(675)	VGGVGLRLIIFAVLSIVNRVRCYSPLS	FQTLTPSPRGPDRLGGIEEEGG			
TV1.8_2	(693)	VGGVGLRLIIFAVLSIVNRVRCYSPLS	FQTLTPSPRGPDRLGGIEEEGG			
TV1.8_5	(695)	VGGVGLRLIIFAVLSIVNRVRCYSPLS	FQTLTPSPRGPDRLGGIEEEGG			
TV2.12-5/1	(680)	VGGVGLRLIIFAVLSIVNRVRCYSPLS	FQTLTPSPRGPDRLGGIEEEGG			
Consensus	(701)	VGGVGLRLIIFAVLSIVNRVRCYSPLS	FQTLTPSPRGPDRLGGIEEEGG			

FIGURE 2B

		751		800
SF162	(725)	ERDRDRSSPFWHLLIATIWDLRSLGLFSYHRGRDLALTAAIRIVELGR-		
TV1.8_2	(743)	EQDRDRSIRLVSGFISHANDMRNLCHFSYHRIRDFILLAVRAVELLGH		
TV1.8_5	(745)	EQDRDRSIRLVSGFISHANDMRNLCHFSYHRIRDFILLAVRAVELLGH		
TV2.12-5/1	(730)	EQSSRSIRLVSGFISHANDMRNLCHFSYHRIRDFILLAVRAVELLGH		
Consensus	(751)	EQDRDRSIRLVSGFISLAWDDLRLCLFSYHRLRDFILLAVRAVELLGH		
		801		850
SF162	(774)	-----RGWEALKYWGNTLQYWIQELKNSAVSLFSAATATAVAEGTDRIE		
TV1.8_2	(793)	SLRGLOQGWELKYLGLSLVQYWGLELKKSAISLLDTTAAIAVAEGTDRIE		
TV1.8_5	(795)	SLRGLOQGWELKYLGLSLVQYWGLELKKSAISPLDTTAAIAVAEGTDRIE		
TV2.12-5/1	(780)	SLRGLOQGWELKYLGLSLVQYWGLELKKSAISLLDTTAAIAVAEGTDRIE		
Consensus	(801)	SLRGLOQGWELKYLGLSLVQYWGLELKKSAISLLDTTAAIAVAEGTDRIE		
		851		876
SF162	(818)	LVQRICRAFLHIFRRIRQGFRAALL-		
TV1.8_2	(843)	LVQRICRAFLHIFRRIRQGFRAALL-		
TV1.8_5	(845)	LVQRICRAFLHIFRRIRQGFRAALL-		
TV2.12-5/1	(830)	LVQRICRAFLHIFRRIRQGFRAALL-		
Consensus	(851)	LVQRICRAFLHIFRRIRQGFRAALL-		

FIGURE 2C

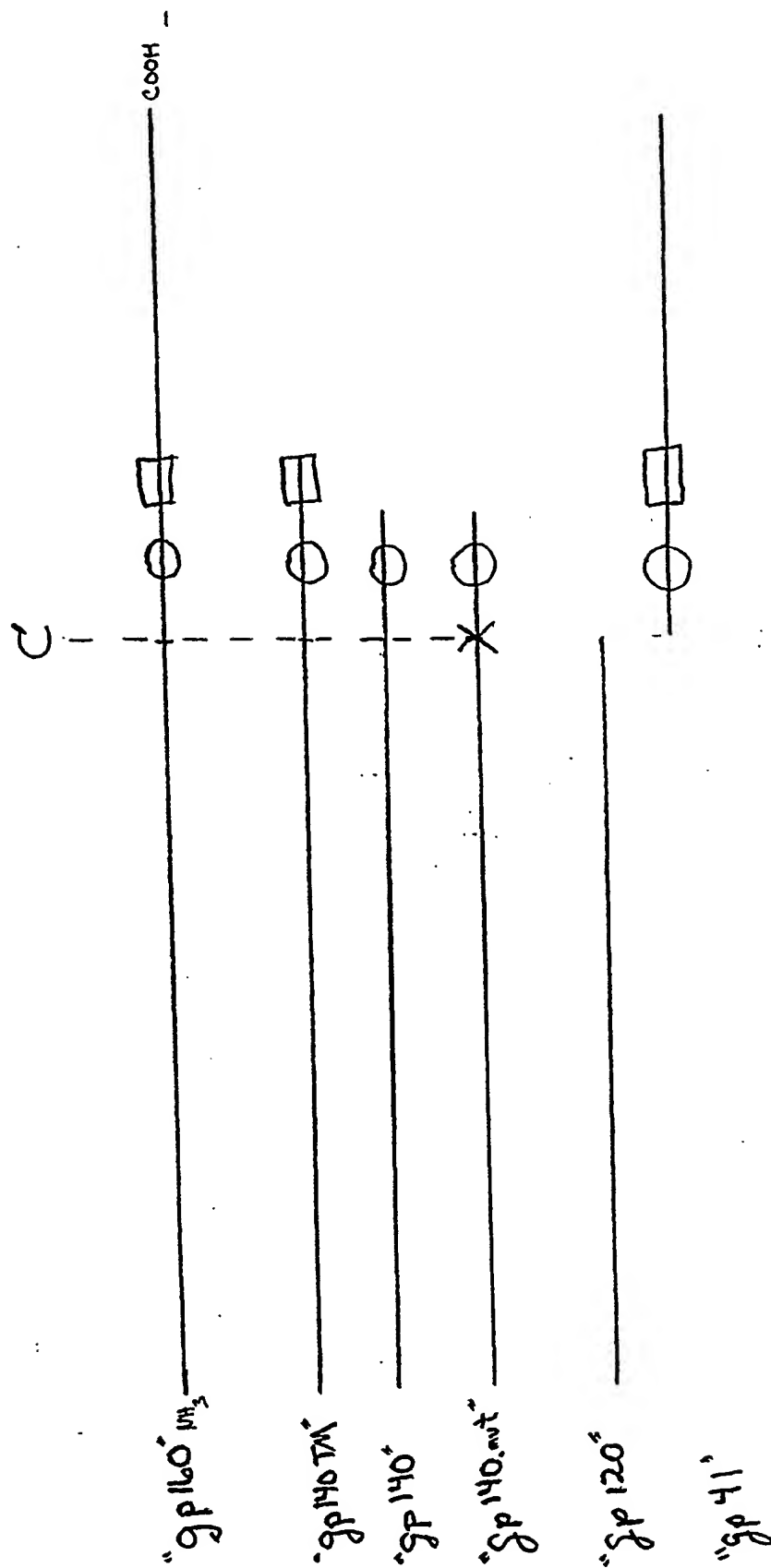


FIG. 3

Figure 4

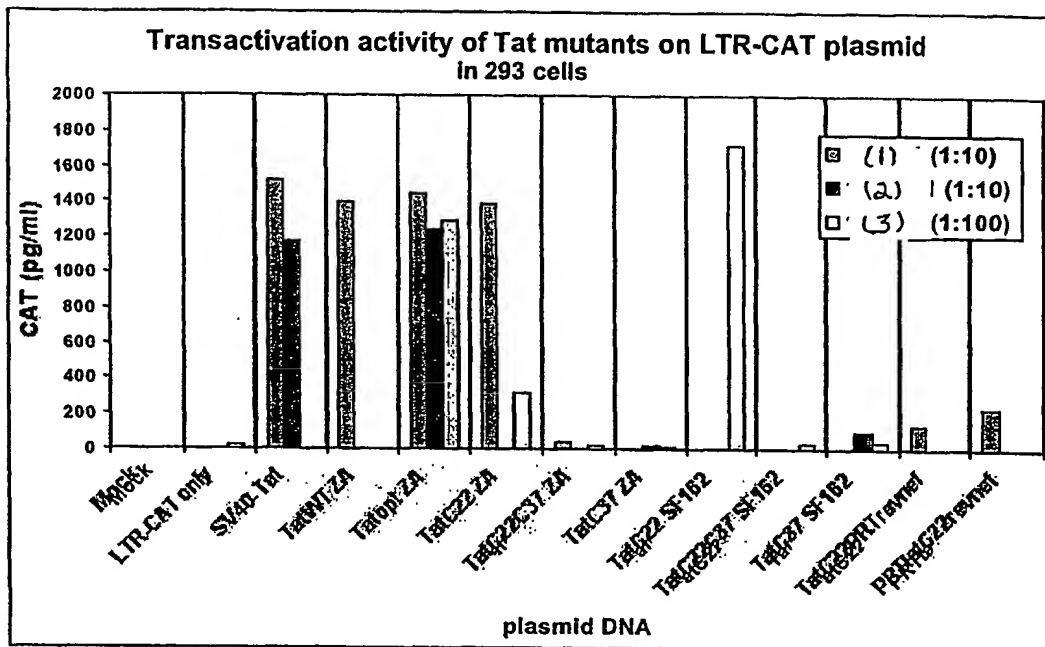


Figure 5

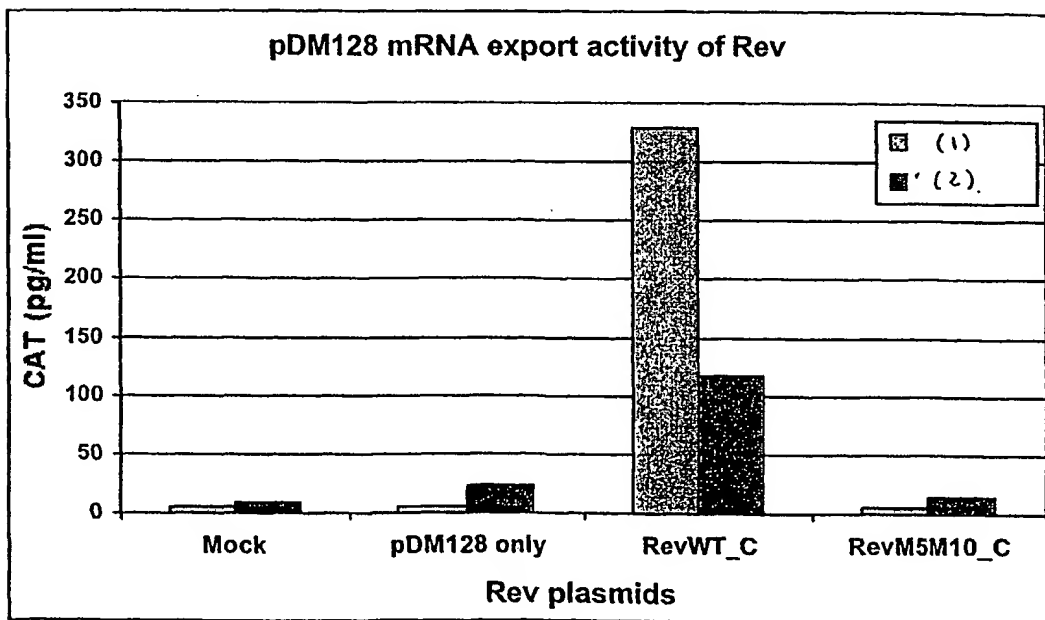


Figure 6
(Sheet 1 of 2)

GagComplPolmut_C

GCCACCATGGGCGCCCCGCGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCTGGGAGCGCATCCGCTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTC
GCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCGCC
CTGCAGACCGCGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCTGTACTGCTGCACGAG
AAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTG
CAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCTGAACGCCCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACCCCCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAG
GAGGCCGCCGAGTGGGACCGCTGCACCCCGTGCACGCCGGCCCCATCGCCCCGGCCAGATGCGCGAG
CCCCCGCGGACATCGCCCGCACACACAGCACCTTGCAGGAGCAGATCGCTGGATGACCAGCAAC
CCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCTGAACAAGATCGTGCAGATG
TACAGCCCCGTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAACTGGATGACCGACACCTGCTG
GTGAGAAGCCAAACCCCGACTGCAAGACCATCTGCGCGCTCTCGGCCCGCGCCAGCTGGAGGAG
ATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCTGCTGGCCGAGGCGATGAGC
CAGGCCAACACAGCGTGATGATGAGAAGAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCTTC
AACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCGCCCCGCAAGAAGGGCTGCTGGAAGTGC
GGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCC
AGCCACAAGGGCGCCCCGGCAACTTCTGTCAGAGCCGCCCCGAGCCACCGCCCCCGCGGAGAGC
TTCCGCTTCGAGGAGACCACCCCGCGCAGAAGCAGGAGAGCAAGGACCGCGAGACCTGACCAGCCTG
AAGAGCCTGTTCCGGCAACGACCCCCCTGAGCCAAGAACTTCGCGGAGGCCATGAGCCAGGCCACAGCGCC
AACATCTGATGCAGCGCAGCAACTTCAAGGGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAG
GAGGGCCACATCGCCCGCAACTGCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGCAGGAGGGC
CACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCAGGGC
AAGGCCCGCGAGTTCCCCAGCGAGCAGAACCAGGCCAACAGCCCCACAGCCGCGAGCTGCAGGTGCGC
GGCGACAACCCCGCAGCGAGGCGCGCGAGCGCCAGGGCACCTGAAGTTCCTCCAGATCACCCTG
TGGCAGCGCCCCCTGTTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGACACCGCGCC
GACGACACCGTGTGAGGAGATGAGCCTGCCCGGCAAGTGAAGCCCAAGATGATCGCGGCCATCGGC
GGCTTCATCAAGGTGCGCCAGTACGACCAGATCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACC
GTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCTG
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AAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAG
GGCAAGATCAACCAAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGAC
AGCACCAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTG
CAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGCGTGACCGTGTGGACGTGGGCGAC
GCCTACTTCAGCGTGCCCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTCACCATCCCCAGCATCAAC
AACGAGACCCCGGCATCCGCTACAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATC
TTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCGCAACCCGAGATCGTGATCTACCAG
GCCCCCTGTACGTGGGCGAGCAGCTGGAGATCGGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAG
CACCTGCTGCGCTGGGGCTTACCAACCCCGACAAGAAGCACCAAGGAGCCCCCTTCTGCCCCATC
GAGCTGCACCCGACAAGTGGACCGTGCAGCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAAC
GACATCCAGAAGCTGGTGGGCAAGCTGAAGTGGGCCAGCCAGATCTACCCGGCATCAAGGTGCGCCAG
CTGTGCAAGCTGCTGCGCGGCCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTG
GAGCTGGCCGAGAACCAGGAGATCCTGCGCGAGCCGTCACGGCGTGTAACGACCCAGCAAGGAC
CTGGTGGCCGAGATCCAGAAGCAGGGCCACGACAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAG
AACCTGAAGACCGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAG
GCCGTGCAGAAGATCGCCATGAGAGCATCGTGATCTGGGCAAGACCCCCAAGTTCGCTGCCCCATC
CAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTC
GTGAACACCCCCCTGTTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACC
TTCTACGTGGACGGCGCCACCGCGAGACCAAGATCGGCAAGGCCGCTACGTGACCGACCGGGGCG
CGGCAAGAATCGTGAGCCTGACCGAGACCAACCAAGAGACCGAGCTGCAGGCCATCCAGCTGGCC
CTGCAGGACAGCGCGAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCAGGCC
CAGCCCCACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTG

Figure 6
(Sheet 2 of 2)

TACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAG
GGCATCCGCAAGGTGCTGTTCCCTGGACGGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGAC
CTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCGGGGCTAGCACCGGTTCTAGA

Figure 7
(Sheet 1 of 2)

GagComplPolmutAtt_C

GCCACCATGGGCGCCCCGCGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGC
ATCCGCTGCGCCCCGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGC
CGCGAGCTGGAGAAGTTCGCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAG
CAGATCATCCGCCAGCTGCACCCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTG
TTCAACACCGTGGCCACCCTGTACTGCGTGCACGAGAAGATCGAGGTCCGCGACACCAAG
GAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAGAAGATCCCCAGCAGC
CGAGGCCCGACAAGGGCAAGGTGAGCCAGAACTACCCCATCTGTCAGAACCTGCAGG
GCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCTGAACGCCTGGGTGAAGGTGATCG
AGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCCGCCCTGAGCGAGGGCGCCA
CCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAAGCCCGCATGCAGA
TGCTGAAGGACACCATCAACGAGGAGGCCGCGAGTGGGACCGCGTGCACCCCGTGCAC
GCCGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCACC
ACCAGCACCTGCAGGAGCAGATCGCCTGGATGACCAGCAACCCCCCATCCCCGTGGC
GACATCTACAAGCGGTGGATCATCCTGGGCTGAACAAGATCGTGGCGATGTACAGCCCC
GTGAGCATCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAAGTGGATGACCGAC
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GGCGCCAGCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCCAGCCAAA
GGCCCGCTGCTGGCCGAGGCGATGAGCCAGGCCAACACCAGCGTGATGATGCAGAAAG
GCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACA
TCGCCCGCAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGCAGGCAAGGAGGC
CACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCCTGGGCAAGATCTGGCCCCAGC
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GAGAGCTTCCGCTTCGAGGAGACCACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGA
GACCCTGACCAGCCTGAAGAGCCTGTTCGGCAACGACCCCTGAGCCAAGAATTGCCGA
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CAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCG
CGCCCCCGCAAGAAGGGCTGCTGGAAGTGCAGGCAAGGAGGGCCACCAGATGAAGGACT
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CCCTGCTGGACTCCGCGCGCCGACGACACCGTGTGAGGAGATGAGCCTGCCCGGCAAGT
GGAAGCCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCA
TCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGCCCCACCCCG
TGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCCTGAACTTCCCCATCA
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AGTGGCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAG
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GCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAA
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GCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACT
GGGCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGCG
CCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGA
ACCGCGAGATCCTGCGCGAGCCCGTGCACGCGGTGTACTACGACCCAGCAAGGACCTGG
TGGCCGAGATCCAGAAGCAGGGCCACGACAGTGGACCTACCAGATCTACCAGGAGCCCT

Figure 7
(Sheet 2 of 2)

TCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCCACACCAACGACGTG
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CTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCGTGAACACCCCCCCCCTGGTGAAGCT
GTGGTACCAGCTGGAGAAGGAGGCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGC
CGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCCGCGAGA
AGATCGTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTG
GCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGG
CATCATCCAGGCCAGCCCGACAAGAGCGAGAGCGAGCTGGTGAACCAGATCATCGAGC
AGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGC
GGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTGTTCTGGAC
GGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGC
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Figure 8
(Sheet 1 of 2)

GagComplPolmutIna_C

GCCACCATGGGCGCCCCGCGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGC
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CAGATCATCCGCCAGCTGCACCCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTG
TTCAACACCGTGGCCACCCTGTACTGCGTGCAGGAGAAGATCGAGGTCCGCGACACCAAG
GAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAAGTGCCAGCAGAAGATCCAGCAGGC
CGAGGCCCGCCGACAAGGGCAAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGG
GCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGATCG
AGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCA
CCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGA
TGCTGAAGGACACCATCAACGAGGAGGCCGCGGAGTGGGACCGCGTGCACCCCGTGCAC
GCCGCCCCCATCGCCCCGCGCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGCGACC
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GTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
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ACCCGTGCTGGTGCAGAACGCCAACCCGACTGCAAGACCATCCTGCGCGCTCTCGGCCCC
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CACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCCAGC
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ATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAA
GCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCTGAAGAA
GAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTACTTCAGCGTGCCCTGGACGA
GGACTTCCGCAAGTACACCGCCTTACCATCCCCAGCATCAACAACGAGACCCCGGCAT
CCGCTACCAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAG
CAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCCGAGATCGTGATCTACCA
GGCCCCCTGTACGTGGGCGAGCGACCTGGAGATCGGCCAGCACCGCGCCAAGATCGAGGA
GCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCGACAAGAAGCACCAGAAGGA
GCCCCCTTCTGCCCATCGAGCTGCACCCCGACAAGTGGACCGTGACGCCATCGAGCT
GCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACT
GGGCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCG
CCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGA
ACCGCGAGATCCTGCGCGAGCCCGTGACGGCGTGTACTACGACCCAGCAAGGACCTGG
TGGCCGAGATCCAGAAGCAGGGCCACGACCAAGTGACCTACCAGATCTACCAGGAGCCCT
TCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTG

Figure 8
(Sheet 2 of 2)

AAGCAGCTGACCGAGGCCGTGCAGAAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAA
GACCCCCAAGTTCCGCCTGCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTA
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GTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGC
CGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCCGCGCAGA
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GCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGG
CATCATCCAGGCCAGCCGACAAGAGCGAGAGCGAGCTGGTGAACCAGATCATCGAGC
AGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGC
GGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTGTTCTGGAC
GGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGC
GGCCCTAGGATCGATTAAAAGCTTCCCGGGGCTAGCACCGGTTCTAGA

Figure 9
(Sheet 1 of 2)

GagComplPolmutInaTatRevNef_C

GCCACCATGGGCGCCCGCCAGCATCCTGCGCGGCGCAAGCTGGACGCCTGGGAGCGCATCCGCTG
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GCCCTGAACCCCGGCTGTCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCCTGTACTGCTGTCACGAG
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AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTG
CAGGGCCAGATGGTGCACAGGCCATCAGCCCCCGCACCTGAACGCCCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACCCCCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAG
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CCCCCATCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCCCTGAACAAGATCGTGCAGGATG
TACAGCCCCGTGAGCATCTTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAATCGATGACCGACACCCCTGCTG
GTGCAGAACGCCAACCCCGACTGCAAGACCATCTGCGCGCTCTCGGCCCGCGGCCAGCCTGGAGGAG
ATGATGACCGCCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGTGGCCGAGGCGATGAGC
CAGGCCAACACCAGCGTGTATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTCTTC
AACTGCGGCAAGGAGGCCACATCGCCCGCAACTGCGCGGCCCTGCAGGAGCAGATCGCTCAAGTCTTC
GGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCAGGGC
AGCCACAAGGGCGCCCCGCGCAACTTCTTGCAGAGCGCCCCGAGCCACCGCCCCCGCGCGAGAGC
TTCCGCTTTCAGGAGACACCCCGGCCAGAAAGCAGGAGAGCAAGGACCGCGAGACCCCTGACCGAGCCTG
AAGAGCCTGTTCCGCAACGACCCCTGAGCCAAGAATTCGCGGAGGCCATGAGCCAGGCCACCAGCGCC
AACATCTGTATGCAGCGCAGCAACTTCAAGGGCCCCAAGCGCATCATCAAGTGTCTTCAACTGCGGCAAG
GAGGGCCACATCGCCCGCAACTGCGCGGCCCGCCGCAAGAAGGGCTGCTGGAAGTGCAGGCAAGGAGGC
CACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCAGGGC
AAGGCCCGCGAGTTCCCCAGCGAGCAGAACCGCGCCAACAGCCCCACCGCCGAGCTGCAGGTGCGC
GGCGACAACCCCGCAGCGAGGCCGCGCGGAGCGCCAGGGCACCTGAACCTCCCCCAGATCACCCCTG
TGGCAGCGCCCCCTGGTGTAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGCGCC
GACGACACCGTGTGGAGGAGATGAGCCTGCCCGCAAGTGGGAAGCCCAAGATGATCGCGGCCATCGGC
GGCTTTCATCAAGGTGCGCCAGTACGACCAGATCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACC
GTGCTGTATCGGCCCAACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCCCTG
AACTTCCCCATCAGCCCCATCGAGACCGTGCCTGTGAAGCTGAAGCCCGCATGGACGGCCCCAAGGTG
AAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAG
GGCAAGATCACCAAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGAC
AGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTG
CAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGCGTGACCGTGTGGACGTGGGCGAC
GCCTACTTCAGCGTGGCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTACCATCCCCAGCATCAAC
AACGAGACCCCCGGCATCCGCTACCAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATC
TTCCAGAGCAGCATGACCAAGATCTTGGAGCCCTTCCGCGCCGCAACCCCGAGATCGTGATCTACCAG
GCCCCCTGTACGTGGGCAGCGACCTGGAGATCGGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAG
CACCTGTGCGCTGGGGCTTACCACCCCGACAAGAAGCACCAAGGAGCCCCCTTCTGCCCCATC
GAGCTGCACCCCGACAAGTGGACCGTGCAGCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAAC
GACATCCAGAAGCTGGTGGGCAAGCTGAACCTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAG
CTGTGCAAGCTGTGCGCGGCCCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTG
GAGCTGGCCGGAAGAACCGCGAGATCTTGCAGGAGCCGTCACGGCGTGTACTACGACCCAGCAAGGAC
CTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAG
AACCTGAAGACCGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAG
GCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCGCGCTGCCCATC
CAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTC
GTGAACACCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCCCGGAGACC
TTCTACGTGGACGGCGCCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGC
CGGCAAGAAGATCGTGAGCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCC
CTGCAGGACAGCGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCC
CAGCCCCACAAGAGCGAGAGCGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTG

Figure 9
(Sheet 2 of 2)

TACCTGAGCTGGGTGCCCCCACAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAG
GGCATCCGCAAGGTGCTGTTCTTGACGGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGAC
CTGTACGTGGGCAGCGGCGGCCCTAGGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCACCCCGGC
AGCCAGCCCAAGACCGCGGCAACAAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTC
CAGACCAAGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCGCAGCGCCGAGCGCCCCCCCCAGC
AGCGAGGACCACCAGAACCCCATCAGCAAGCAGCCCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGC
GAGGAGAGCAAGAAGAAGGTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGCCGAGC
GGCGACAGCGACGAGGCCCTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTAC
CCCAAGCCCGAGGGCACCCGCCAGGCCGACCTGAACCGCCGCGCGCGCTGGCGCGCCCGCCAGCGCCAG
ATCCACAGCATCAGCGAGCGCATCCTGAGCACCTGCCTGGGCGCGCCCGCGAGCCCGTGCCCTTCCAG
CTGCCCCCGACCTGCGCCTGCACATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGC
CAGGGCACCAACCGAGGCGTGCGCAGCCCCCTCGAGGCCGCAAGTGGAGCAAGAGCAGCATCGTGGGC
TGGCCCGCGTGCGCGAGCGCATCCGCCGCAACGAGCCCGCGCGCGAGGGCGTGGGCGCCGCCAGCCAG
GACCTGGACAAGCACGGCGCCCTGACCAGCAGCAACACCGCCGCAACAACGCCGACTGCGCCTGGCTG
GAGGCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGTGCCCCCTGCGCCCCATGACC
TACAAGGCCGCCTTCGACCTGAGCTTCTTCTGAAGGAGAAGGGCGGCTGGAGGGCCTGATCTACAGC
AAGAAGCGCCAGGAGATCCTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGGCTGGCAGAAC
TACACCCCGGCCCCGGCGTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGAC
CCCCGCGAGGTGGAGGAGGCAACAAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGC
ATGGAGGACGAGGACCGCGAGGTGCTGAAGTGGAAGTTGACAGCAGCCTGGCCCGCGCCACATGGCC
CGCGAGCTGCACCCCGAGTACTACAAGGACTGCGCCTAA

Figure 10
(Sheet 1 of 1)

GagPolmut_C

GCCACCATGGGCGCCCGGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGCATCCGCCTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTC
GCCCCGAACCCCGGCCCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCTGTACTGCGTGACGAG
AAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAATACCCCATCGTGACAGAACCTG
CAGGGCCAGATGGTGCACACAGCCATCAGCCCCCGCACCCCTGAACGCCCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTTCAGCCCCGAGGTGATCCCCATGTTTACCGCCCTGAGCGAGGGCGCCACCCCCCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACAGGCCCATGTCAGATGCTGAAGGACACCATCAACGAG
GAGGCCGCGAGTGGGACCGGTGCACCCCGTGCACGCGGCCCATCGCCCCGGCCAGATGCGCGAG
CCCCGCGGCGACGACATCGCCGGCACACCAGCACCCCTGCAGGAGCAGATCGCCTGGATGACCAGCAAC
CCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCCCTGAACAAGATCGTGCGGATG
TACAGCCCCGTGAGCATCCTGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTAGCTGGACCGC
TTCTTCAAGACCCCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAATGGATGACCGACACCCCTGCTG
GTGCAGAACGCCAACCCGACTGCAAGACCATCCTGCGCGCTCTCGGCCCCGGCGCCAGCCTGGAGGAG
ATGATGACCGCTGCCAGGGCGTGGGCGGCCACACAGGCCCGCGTGTCTGGCCGAGGCGATGAGC
CAGGCCAACACAGCGTGATGATGCAGAAAGCAACTTTAAAAAGGGCCCCAAGCGCATCATCAAGTGC
TTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCCCCCCCCGCAAGAAGGGTGTCTGGAAG
TGCGGCAAGGAGGGCCACAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTG
GCCTTCCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAACCAGCGCCAACAGCCCCACCAGCCGC
GAGCTGCAGGTGCGCGGCGACAACCCCCGAGCGAGGCCGCGCGGAGCGCCAGGGCACCCCTGAAC TTC
CCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTG
CTGGACACCGCGCCGACGACACCGTGCTGGAGGAGATGAGCCTGCCCGCAAGTGGAAGCGCTGAAGATG
ATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAG
AAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAG
CTGGGCTGCACCTGAAC TTCCCATCAGCCCCATCGAGACCGTGCCCGTGAAGCTGAAGCCCGGCATG
GACGGCCCCAAGGTGAAGCAGTGGCCCCGTACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAG
GAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCGGAGAACCCCTACAACACCCCCGTGTTCCGCC
ATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGACTTCCGCGAGCTGAACAAGCGCACCCAG
GACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGCCCTGAAGAAGAAGAAGCGCTGACCGTG
CTGGACGTGGGCGACGCTACTTCAGCGTGGCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTCACC
ATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGCTGCCCCAGGGCTGGAAG
GGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCCGAG
ATCGTGATCTACCAGGCCCCCTGTACGTGGGCGAGCAGCTGGAGATCGGCCAGCACCGGCCCAAGATC
GAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCCGACAAGAAGCACCAGAAGGAGCCC
CCCTTCTGCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCGAGCTGCCCGAGAAGGAG
AGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAAC TGGGCCAGCCAGATCTACCCCGGC
ATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGCGCCAAAGGCCCTGACCGACATCGTGCCCTGACC
GAGGAGGCCGAGCTGGAGCTGGCCGAGAACC GCGAGATCCTGCGCGAGCCCGTGACGGCGTGTA CTAC
GACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAGTGGACCTACCAGATCTAC
CAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTG
AAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAG
TTCCGCTGCCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATC
CCCGAGTGGGAGTTCTGTAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATC
ATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTAC
GTGACCGACCGGGGCGGCGAGAAGATCGTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAG
GCCATCCAGCTGSCCTG CAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTG
GGCATCATCCAGGCCAGCCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAGCTGATC
AAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCACAAGGGCATCGGCGGCAACGAGCAGATCGAC
AAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTGTTCTCTGGACGGCATCGATGGCGGCATCTGATCTAC
CAGTACATGGACGACCTGTACGTGGGCGAGCGCGGCCCTAGGATCGATTAAAAGCTTCCCCGGGCTAGC
ACCGGTTCTAGA

Figure 11
(Sheet 1 of 1)

GagPolmutAtt_C

GTTCGACGCCACCATGGGCGCCCGCGCCAGCATCCTGCGCGCGGCAAGCTGGACGCCTGGGAGCGCATC
CGCCTGCGCCCCCGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAG
AAGTTTCGCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCAC
CCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCTGTACTGCGTG
CACGAGAAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGC
CAGCAGAAGATCCAGCAGGCCGAGGCCCGGACAAGGGCAAGGTGAGCCAGAATAACCCATCGTGCAG
AACCTGCAGGGCCAGATGGTGCACCGGCCATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGATC
GAGGAGAAGGCCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACCCCCAG
GACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCCATGTCAGATGCTGAAGGACACCATC
AACGAGGAGGCCCGGAGTGGGACCGCGTGCACCCCGTGCACGCCGCGCCCATCGCCCCCGGCCAGATG
CGCGAGCCCCGCGGCAGCGACATCGCCGGCACCACAGCACCCCTGCAGGAGCAGATCGCCTGGATGACC
AGCAACCCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCCCTGAACAAGATCGTG
CGATGTACAGCCCCGTGAGCATCTTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTG
GACCGCTTCTTCAAGACCCTGCGCGCCGAGCAGACCCAGGAGGTGAAGAACTGGATGACCGACACC
CTGCTGGTGCAGAACGCCAACCCGACTGCAAGAACCATCTGCGCGCTCTCGCCCCCGGCCAGCCTG
GAGGAGATGATGACCGCTGCCAGGGCGTGGGCGCCCCAGCCACAAGGCCCGCGTGGCCGAGGCC
ATGAGCCAGGCCAACACCAGCGTATGATGCAGAAGAGCAACTTTAAAAAGGGCCCCAAGCGCATCATC
AAGTGCTTCACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCGCGCCCCCGCAAGAAGGGCTGC
TGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAG
GACCTGGCCTTCCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAACCGCGCCAACAGCCCCACC
AGCCGCGAGCTGCAGGTGCGCGCGGACAAACCCCGCAGCGAGGCCGCGCGCGAGCGCCAGGGCACCCCTG
AACTTCCCCCAGATCACCCCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAG
GCCCTGCTGGACTCCGGCGCCGACGACACCGTGTGGAGGAGATGAGCCTGCCCGGCAAGTGAAGCCCC
AAGATGATCGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGC
GGCAAGAAGGCCATCGGCACCCGTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTG
ACCCAGCTGGGCTGCACCCTGAACCTTCCCCATCAGCCCCATCGAGACCGTGCCTGTAAGCTGAAGCCC
GGCATGGACGCCCCCAAGGTGAAGCAGTGGCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATC
TGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCGGAGAACCCCTACAACACCCCGTG
TTCCGCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGAGCTTCCGCGAGCTGAACAAGCGC
ACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGCCCTGAAGAAGAAGAGCGTG
ACCGTGCTGGACGTGGGCGACGCTTACTTACGCGTGGCCCTGGACGAGGACTTCCGCAAGTACACCGCC
TTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAAGTACAACGTGCTGCCCCAGGGC
TGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAAC
CCCGAGATCGTGATCTACCAAGCCCCCTGTACGTGGGCAGCGACCTGGAGATCGGCCAGCACCGCGCC
AAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCGGACAAGAAGCACCAGAAG
GAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCGAGCTGCCCGAG
AAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACCTGGGCCAGCCAGATCTAC
CCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGCGCCAAGGCCCTGACCGACATCGTGCCC
CTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGCGCGAGCCCGTGCACGGCGTG
TACTACGACCCACGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAG
ATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAAC
GACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACC
CCCAAGTTCCGCTGCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACC
TGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCTGGTGAAGCTGTGGTACCAGTGGAGAAGGAG
CCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCAACCGCGAGACCAAGATCGGCAAGGCC
GGCTACGTGACCGACCGGGGCCGAGAGATCGTGAGCCTGACCGAGACCACCAACAGAAGACCGAG
CTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTAC
GCCCTGGGCATCATCAGGCCAGCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAG
CTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCACAAGGGCATCGGCGGCAACGAGCAG
ATCGACAAGCTGGTGAAGGAGCATCCGCAAGGTGCTGTTCTTGGACGGCATCGATGGCGGCATCGTG
ATCTACCAGTACATGGACGACCTGTACGTGGGCGAGCGCGGCCCTAGGATCGATTAAAGCTTCCCGGG
GCTAGCACCGGTTCTAGA

Figure 12
(Sheet 1 of 1)

GagPolmutIna_C

GTCGACGCCACCATGGGCGCCGCGCCAGCATCTGCGCGGCGGCAAGCTGGACGCCCTGGGAGCGCATC
CGCCTGCGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAG
AAGTTCGCCCCGAACCCCGGCCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCAC
CCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTC AACACCCGTGGCCACCCCTGTACTGCGTG
CACGAGAAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGAACAAGATCGAGGAGGAGCAGAACAAGTGC
CAGCAGAAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAATAACCCCATCGTGCAG
AACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCTGGGTGAAGGTGATC
GAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAACGCCCTGAGCGAGGGCGCCACCCCCAG
GACCTGAACACGATGTTGAACACCGTGGGCGGCCACAGGCCGCCATGCAGATGCTGAAGGACACCATC
AACGAGGAGGCCGCGGAGTGGGACCGCGTGCACCCCGTGCACGCCGCGGCCCATCGCCCCCGGCCAGATG
CGCGAGCCCCCGCGCAGCGACATCGCCCGCACACCAGCACCCCTGCAGGAGCAGATCGCCTGGATGACC
AGCAACCCCCCCATCCCGTGGGCGACATCTACAAGCGGTGGATCATCTTGGGCCCTGAACAAGATCGTG
CGGATGTACAGCCCCGTGAGCATCTTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTG
GACCGCTTCTTCAAGACCCCTGCGCGCCGAGCAGACACCAGGAGGTGAAGAAGTGGATGACCGACACC
CTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCTTGCAGCGCTCTCGGCCCGCGCCAGCCCTG
GAGGAGATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGTGCGCGAGGCG
ATGAGCCAGGCCAACACCAGCGTGATGATGCAGAAGAGCAACTTTAAAAAGGGCCCCAAGCGCATCATC
AAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCCCCCGCAAGAAGGGCTGC
TGGAAGTGC GCGCAAGGAGGGCCACAGATGAAGGACTGCACCGAGCGCCAGGCCAACCTTCTTCCGCGAG
GACCTGGCCCTTCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAACC GCGCCAACAGCCCCACC
AGCCGCGAGATGCGAGGTGCGCGCGCAACAACCCCGCAGCGAGGCCGCGCGCGAGCGCCAGGGCACCCCTG
AACTTCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAAGCATCAAGGTGGGCGGCCAGATCAAGGAG
GCCCTGCTGGCCACCGCGCCGACGACACCGTGCTGGAGGAGATGAGCCTGCCCGGCAAGTGAAGGCC
AAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGC
GGCAAGAAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTG
ACCCAGCTGGGCTGCACCTGAACCTTCCCCATCAGCCCCATCGAGACCGTGCCCGTGAAGCTGAAGCCC
GGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATC
TGCGAGGAGATGGAGAAGGAGGCAAGATCACCAAGATCGGCCCGGAGAACCCTTACAACACCCCGTG
TTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGC
ACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGCGTG
ACCGTGCTGGACGTGGGCGACGCTACTTTCAGCGTGCCTTGGACGAGGACTTCCGCAAGTACACCGCC
TTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAAGTACAACGTGCTGCCCCAGGGC
TGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAAC
CCCGAGATCGTGATCTACCAGGCCCCCTGTACGTGGGCAGCGACCTGGAGATCGGCCAGCACCGCGCC
AAGATCGAGGAGTGC GCAAGCACCTGCTGCGCTGGGGCTTCAACACCCCGACAAGAAGCACCAAGAG
GAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCGAGCTGCCCGAG
AAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAAC TGGGCCAGCCAGATCTAC
CCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCCAAGGCCCTGACCGACATCGTGCCC
CTGACCGAGGAGGCGGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGCGCGAGCCCGTGCACGGCGTG
TACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAG
ATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAAC
GACGTGAAGCAGCTGACCGAGGCGGTGCAAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACC
CCCAAGTTCCGCTTGCCTATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACC
TGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAG
CCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCAACCGCGAGACCAAGATCGGCAAGGCC
GGCTACGTGACCGACCGGGGCCGCGAGAAGATCGTGAGCCTGACCGAGACCAACCAAGAGACCGAG
CTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTAC
GCCCTGGGCATCATCCAGGCCAGCCCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAG
CTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAG
ATCGACAAGCTGGTGAAGCAAGGGCATCCGCAAGGTGCTGTTCTTGGACGGCATCGATGGCGGCATCGTG
ATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGCGGCCCTAGGATCGATTAAAAGCTTCCCGGG
GCTAGCACCGGTTCTAGA

Figure 13
(Sheet 1 of 1)

GagProtInaRTmut_C

GCCACCATGGGCGCCCGGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGCATCCGCCTG
CGCCCCGCGGCCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTTC
GCCCTGAACCCCGGCCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTC AACACCCGTGGCCACCCTGTACTGCGTGCACGAG
AAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGA ACTACCCCATCGTGCAAGACCTG
CAGGGCCAGATGGTGCACCGCCATCAGCCCGCCACCCCTGAACGCCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTACGCCCCGAGGTGATCCCCATGTTTCAACGCCCTGAGCGAGGGCGCCACCCCCAGGACCTG
AACACGATGTTGAACACCCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAG
GAGGCCCGCGAGTGGGACCGCGTGCACCCCGTGCACGCCGCCCATCGCCCCCGCCAGATGCGCGAG
CCCCGCGGCAGCGACATCGCCGCCACCACCAGCACCCCTGCAGGAGCAGATCGCCTGGATGACCAGCAAC
CCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCCCTGAACAAGATCGTGCGGATG
TACAGCCCCGTGAGCATCTTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCCCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAACTGGATGACCGACACCCCTGCTG
GTGCAGAACGCCAACCCCGACTGCAAGACCATCTCTGCGCGCTCTCGGCCCGGCCGCGCAGCCTGGAGGAG
ATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGCCTGGCCGAGGCGATGAGC
CAGGCCAACACCAGCGTGTATGTCAGAAAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCTTTC
AACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGC
GGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCC
AGCCACAAGGGCCCGCCCGGCAACTTCTGCGAGCGCCCGGAGCCACCGCCCCCGCCGAGAGC
TTCCGCTTTCGAGGAGACCACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACCCCTGACCAGCCTG
AAGAGCCTGTTTCGGCAACGACCCCTGAGCCAGCAAGAAATAATCCCCAGATCACCTGTGGCAGCGCCCC
CTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGCGCCGACGACCCGTG
CTGGAGGAGATGAGCCTGCCCGGCAAGTGAAGCCCAAGATGATCGGCGGCATCGGCGGCTTTCATCAAG
GTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGC
CCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCTGA ACTTCCCCATC
AGCCCCATCGAGACCGTGGCCGTGAAGCTGAAGCCCGGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCC
CTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACC
AAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGG
CGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGTGGGCATC
CCCCACCCCGCCGGCTGAAGAAGAAGAAGAGCGTGACCGTGTGACGTTGGGCGACGCCCTACTTTCAGC
GTGCCCCCTGGACGAGGACTTCCGCAAGTACACCGCTTCAACATCCCCAGCATCAACAACGAGACCCCC
GGCATCCGCTACCACTACAACGTGTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGC
ATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCCGAGATCGTGATCTACCAGGCCCCCTGTAC
GTGGSCAGCGACCTGGAGATCGGCCAGCACCGCGCCCAAGATCGAGGAGCTGCGCAAGCACCTGTGCGC
TGGGGCTTCAACACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGCCCCATCGAGCTGCACCCC
GACAAGTGGACCGTGCAGCCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAG
CTGGTGGGCAAGCTGA ACTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTG
CTGCGCGGCGCAAGGCCCTGACCGACATCGTGCCCCGAGCGAGGAGGCGGAGCTGGAGCTGGCCGAG
AACC CGAGATCTGCGCGAGCCCGTGCACGGCGTGTACTACGACCCAGCAAGGACCTGTTGGCCGAG
ATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACC
GGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAAG
ATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCTGCCCCATCCAGAAGGAGACC
TGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCC
CCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGAC
GGCGCCGCCAACC CGGAGACCAAGATCGGCAAGGCCGCTACGTGACCGACCGGGGCCGCGCAGAAGATC
GTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGC
GGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCCACAAG
AGCGAGAGCGAGCTGTGTAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGG
GTGCCCCGCCACAAGGGCATCGGCGGCCAAGCAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAG
GTGCTCGCTTAA

Figure 14
(Sheet 1 of 2)

GagProtInaRTmutTatRevNef_C

GCCACCATGGGCGCCCGCGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGCATCCGCCCTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTC
GCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCTGTACTGCGTGCACGAG
AAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAATACCCCATCGTGCAGAACCTG
CAGGGCCAGATGGTGCACAGGCCATCAGCCCCCGCACCCCTGAACGCCCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGGCCCTGAGCGAGGGCGCCACCCCCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAG
GAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCACGCCGGCCCCATCGCCCCGGCCAGATGCGCGAG
CCCCCGCGGACGACATCGCCGGCACCACAGCACCCCTGCAGGAGCAGATCGCCTGGATGACCAAGCAAC
CCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCCCTGAACAAGATCGTGGCGGATG
TACAGCCCCGTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAACTGGATGACCGACACCCCTGCTG
GTGCAGAACGCCAACCCCGACTGCAAGACCATCTGCGCGCTCTCGGCCCGCGGCCAGCTGGAGGAG
ATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGACGCCACAAGGCCCGCGTGTGGCCGAGGCGATGAGC
CAGGCCAACACAGCGTGATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCTTC
AACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGC
GGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTGGGCAAGATCTGGCCCC
AGCCACAAGGGCGGCCCGGCAACTTCTTGCAGAGCGCCCCGAGCCACCGCCCCCGGCCGAGAGC
TTCCGCTTCGAGGAGACCACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACCCCTGACAGCCTG
AAGAGCCTGTTTCGCAACGACCCCTGAGCCAGAAAGAATTCCCCCAGATCACCCCTGTGGCAGCGCCCC
CTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGGCGCCGACGACACCGTG
CTGGAGGAGATGAGCCTGCCCGGCAAGTGGAAGCCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAG
GTGCGCCAGTACGACAGATCTCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGC
CCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCCCTGAAGTTCCTCCATC
AGCCCCATCGAGACCGTGCCCGTGAAGCTGAAGCCCGGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCC
CTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCAC
AAGATCGGCCCGGAGAACCCTACAAACCCCCGTGTTTCGCCATCAAGAAGAAGGACAGCACCAAGTGG
CGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATC
CCCCACCCCGCGGCTGAAGAAGAAGAAGAGCGTGACCGTGTGGAAGTGGGCGACGCTTCTCAGC
GTGCCCCTGAGCAGGAGCTTCCGCAAGTACACCGCCTTACCATCCCCAGCATCAACAACGAGACCCCC
GGCATCCGCTACCACTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGC
ATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCGAGATCGTGATCTACCAGGCCCCCTGTAC
GTGGGACGCGACCTGGAGATCGGCCAGCACCGCGCCAGATCGAGGAGCTGCGCAAGCACCTGTGCGC
TGGGGCTTTCACACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGCCCATCGAGCTGCACCCC
GACAAGTGGACCGTGCAGCCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAG
CTGGTGGGCAAGCTGAAGTGGGCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTG
CTGCGCGGCGCAAGGCCCTGACCGACATCGTGCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAG
AACCGCGAGATCTGCGCGAGCCCGTGCACGGCGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAG
ATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACC
GGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAG
ATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCGCGCTGCCCATCCAGAAGGAGACC
TGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCC
CCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACAGTGGAC
GGCGCGGCCAACCGCGAGACCAAGATCGGCAAGGCCGCTACGTGACCGACCGGGGCGCCGAGAGATC
GTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGC
GGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCGACAAG
AGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGG
GTGCCCGCCCAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAG
GTGCTCaagcttgAGCCCGTGGACCCCAACCTGGAGCCCTGGAACACCCCGCGAGCCAGCCCAAGACC
GCCGGCAACAAGTGTCTGCAAGCACTGCAGCTACCACTGCCCTGGTGAGCTTCAGACCAAGGGCTG
GGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCGCAGCGCCCCCCCCAGCAGCGAGGAGACCACAG
AACCCCATCAGCAAGCAGCCCTGCCCCAGACCCGCGCGACCCACCGGCAGCGAGGAGCAAGAAG

Figure 14
(Sheet 2 of 2)

AAGGTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGCCGAGCGGGCGACAGCGACGAG
GCCCTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGC
ACCCGCCAGGCCGACCTGAACCGCCGCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGC
GAGCGCATCCTGAGCACCTGCCTGGGCGCGCCCGCCGAGCCCGTGCCCTTCCAGCTGCCCCCGACCTG
CGCCTGCACATCGACTGCAGCGAGAGCAGCGGCACCGCGGCACCCAGCAGAGCCAGGGCACCACCGAG
GGCGTGGGCAGCCCCCTCGAGGCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCCGCGTGCGC
GAGCGCATCCGCCGCACCGAGCCCGCCGCGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCAC
GGCGCCCTGACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGCCAGGAGGAG
GAGGAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGTGCCCCCTGCGCCCCATGACCTACAAGGCCGCTTC
GACCTGAGCTTCTTCTGAAGGAGAAGGGCGGCCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAG
ATCCTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGCTGGCAGAACTACACCCCCGGCCCC
GGCGTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACCCCCCGGAGGTGGAG
GAGGCCAACAAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGAC
CGCGAGGTGCTGAAGTGGAAGTTCGACAGCAGCCTGGCCCCGCCGCCACATGGCCCCGCGAGCTGCACCCC
GAGTACTACAAGGACTGCGCCTAA

Figure 15
(Sheet 1 of 1)

GagRTmut_C

GCCACCATGGGCGCCCGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCTGGGAGCGCATCCGCTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTC
GCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCCTGTACTGCGTGCACGAG
AAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCGGACAAGGGCAAGGTGAGCCAGAATAACCCATCGTGCAGAACCCTG
CAGGGCCAGATGGTGCACACAGGCCATCAGCCCCCGCACCCCTGAACGCCCTGGGTGAAGGTGATCGAGGAG
AAGGCCCTTACGCCCCGAGGTGATCCCCATGTTTACCGCCCTGAGCGAGGGCGCCACCCCGCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAG
GAGGCCGCGGAGTGGGACCGCTGCACCCCGTGCACGCCGGCCCCATCGCCCCGGCCAGATGCGCGAG
CCCCCGCGGCAGACATCGCCGGCACCAGCACCCCTGCAGGAGCAGATCGCCTGGATGACCAAGCAAC
CCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTCTGGGCCCTGAACAAGATCGTGCAGATG
TACAGCCCCGTGAGCATCTTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCCCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAAGTGGATGACCGACACCCCTGCTG
GTGCAGAACGCCAACCCCGACTGCAGACCATCTGCGCGCTCTCGGCCCGCGCCAGCCCTGGAGGAG
ATGATGACCGCCCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGTGTCGGCGAGGCGATGAGC
CAGGCCAACACACCGTGTGATGTCAGAAGAGCAACTTCAAGGGCCCCCGGCGCATCGTCAAGTGCTTC
AACTGCGGCAAGGAGGGCCACATCGCCCCGCAACTGCGCGCGCCCCCGCAAGAAGGGCTGTGGAAGTGC
GGCAAGGAGGGCCACAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCC
AGCCACAAGGGCGGCCCGGCAACTTCTTGCAGAGCCGCCCCGAGCCACCGCCCCCGCGCGAGAGC
TTCCGCTTCGAGGAGACACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACCCCTGACCGAGCCTG
AAGAGCCTGTTTCGGCAACGACCCCCCTGAGCCAGAAAGAAATTCCTCCATCAGCCCCATCGAGACCGTGC
GTGAAGCTGAAGCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAG
GCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCCGAGAACCC
TACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGC
GAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCTGAAG
AAGAAGAAGAGCGTGACCGTGTGACGCTGGGCGACGCTACTTCAGCGTGGCCCTGGACGAGGACTTC
CGCAAGTACACCGCCCTTACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCACTACAAC
GTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCTCGAGCCC
TTCCGCGCCCCGAACCCCGAGATCGTGATCTACAGGCCCCCCCTGTACGTGGGCAGCGACCTGGAGATC
GGCCAGCACCCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACACACCCCGGAC
AAGAAGCACCAAGAAGGAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCC
ATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAAGTGG
GCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCCAAGGCCCTG
ACCGACATCGTGGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGCGCGAG
CCCGTGCACGGCGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGAC
CAGTGGACCTACAGATCTACAGGAGCCCTTCAAGAACCTGAAGACCGCAAGTACGCCAAGATGCGC
ACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTG
ATCTGGGGCAAGACCCCAAGTTCCGCTGCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGAC
TACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCCCTGGTGAAGCTGTGGTAC
CAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCAACCGCGAGACC
AAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCCGCGAGAAGATCGTGAGCCTGACCGAGACCACC
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ACCGACAGCCAGTACGCCCTGGGCATCATCAGGCCAGCCCCGACAAGAGCGAGAGCGAGCTGGTGAAC
CAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCACAAGGGCATC
GGCGGCAACGAGCAGATCGACAAGCTGGTGAAGGATCCGCAAGGTGCTCTAA

Figure 16
(Sheet 1 of 2)

GagRTmutTatRevNef_C

GCCACCATGGGCGCCCGGCCAGCATCCTGCGCGCGGCAAGCTGGACGCTGGGAGCGCATCCGCCTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGAAGTTC
GCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCCTGTACTGCGTGCACGAG
AAGATCGAGGTCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAAGTGCCAGCAG
AAGATCCAGCAGGCCGAGGCCGCCGACAAGGGCAAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTG
CAGGGCCAGATGGTGCACACAGGCCATCAGCCCCCGCACCTGAACGCTGGGTGAAGGTGATCGAGGAG
AAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACCCCCAGGACCTG
AACACGATGTTGAACACCGTGGGCGGCCACCAGGCCCGCATGCAGATGCTGAAGGACACCATCAACGAG
GAGGCCGCGGAGTGGGACCGCGTGCACCCCGTGCACGCGGCCCATCGCCCCCGGCCAGATGCGCGAG
CCCCCGCGGACGACATCGCCGGCACCACCAGCACCTGCAGGAGCAGATCGCCTGGATGACCAGCAAC
CCCCCATCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCTGAACAAGATCGTGCAGGATG
TACAGCCCCGTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGC
TTCTTCAAGACCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAACTGGATGACCGACACCTGCTG
GTGCAGAACGCCAACCCCGACTGCAAGACCATCTGCGCGCTCTCGGCCCGCGGCCAGCTGGAGGAG
ATGATGACCGCTGCCAGGGCGTGGGCGGCCCGCCAGCCACAAGGCCCGCGTGTGGCCGAGGCGATGAGC
CAGGCCAACACACGCGTGATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCCTTC
AACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCCCCCGCAAGAAGGGTGTCTGGAAGTGC
GGCAAGGAGGGCCACAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCC
AGCCACAAGGGCCGCCCCGGCAACTTCTGACAGCGCGCCCGAGCCACCGCCCCCGCGGAGAGC
TTCCGCTTCGAGGAGACACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACCTGACACGCTG
AAGAGCCTGTTCGGCAACGACCCCTGAGCCAGAAAGAATTCCCCATCAGCCCCATCGAGACCGTGC
GTGAAGCTGAAGCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCTGACCGAGGAGAAGATCAAG
GCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAGATCGGCCCGGAGAACCC
TACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGC
GAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCTGAAG
AAGAAGAAGAGCGTGACCGTGTGGACGTGGCGGACGCTTACTTACGCGTGCCTTGGACGAGGACTTC
CGCAAGTACACCGCCTTACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAAGTACAAC
GTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCC
TTCCGCGCCCGCAACCCCGAGATCGTGATCTACCAGGCCCTGTACGTGGGCAGCGACCTGGAGATC
GGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCGGAC
AAGAAGCACCAAGAGAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCC
ATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAAGTGG
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CAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCCTGAAGACCGGCAAGTACGCCAAGATGCGC
ACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTG
ATCTGGGGCAAGACCCCAAGTTCCGCTGCCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGAC
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CAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACTTGAAGCTGGGTGCCCCGCCCCACAAGGGCATC
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GACCCCAACCTGGAGCCCTGGAACACCCCGGCGAGCCAGCCCAAGACCGCGGCAACAAGTGTACTGC
AAGCACTGCAGCTACCACTGCCTGGTGAAGTTCAGACCAAGGGCCTGGGCATCAGCTACGGCCGCAAG
AAGCGCCGCGAGCGCGCAGCGCCCCCGGCGAGCGAGGAGCAAGAAGAAGTGGAGAGCAAGACCGAG
CTGCCCCAGACCCGCGGCGACCCACCGGCGAGCGAGGAGCAAGAAGAAGTGGAGAGCAAGACCGAG
ACCGACCCCTTTCAGCCCGGGGCGGCGGCGAGCGGCGAGCGACGAGGCCCTGCTGCAGGCGGTGCGC
ATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCGAGGGCACCCGCGAGGCCGACCTGAAC
CGCCGCGCGCTGGCGCGCCCGGCGAGCGCGAGATCCACAGCATCAGCGAGCGCATCCTGAGCACCTGC
CTGGGCCGCCCCGCGAGCCCGTGCCTTCCAGCTGCCCCCGACCTGCGCTGCACATCGACTGCAGC

Figure 16
(Sheet 2 of 2)

GAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCAACGAGGGCGTGGGCAGCCCCCTCGAG
GCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGTGCGCGAGCGCATCCGCCGCACCGAG
CCCGCCGCCGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCACGGCGCCCTGACCAGCAGCAAC
ACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCC
GTGCGCCCCCAGGTGCCCCCTGCGCCCCATGACCTACAAGGCCGCCCTTCGACCTGAGCTTCTTCCTGAAG
GAGAAGGGCGGCCCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATCCTGGACCTGTGGGTGTAC
CACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCGGCCCGGCGTGGCTACCCCTGACC
TTCGGCTGGTGCCTCAAGCTGGTGGCCCGTGGACCCCGCGAGGTGGAGGAGGCCAACAAGGGCGAGAAC
AACTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGCGAGGTGCTGAAGTGGAAG
TTCGACAGCAGCCTGGCCCGCCGCCACATGGCCCGCGAGCTGCACCCGAGTACTACAAGGACTGCGCC
TAA

Figure 17
(Sheet 1 of 1)

GagTatRevNef_C

GCCACCATGGGCGCCCGGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGCATCCGCTTG
CGCCCCGGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCGCAGCCGCGAGCTGGAGAAGTTC
GCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCCGCC
CTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCTGTACTGCGTGCACGAG
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AAGATCCAGCAGGCCGAGGCCGCGGACAAGGGCAAGGTGAGCCAGAATAACCCCATCGTGCAGAACCTG
CAGGGCCAGATGGTGCACCAGGCCATCAGCCCCGACCCCTGAACGCCTGGGTGAAGGTGATCGAGGAG
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CAGGCCAACACCAGCGTGATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCATCGTCAAGTGCTTC
AATGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGC
GGCAAGGAGGGCCACAGATGAAGGACTGCACCGAGCGCCAGGCCAATTCCTGGGCAAGATCTGGCCC
AGCCACAAGGGCGCCCCGCAACTTCCTGCAGAGCCGCCCCGAGCCACCGCCCCCGCGCGAGAGC
TTCGCTTCGAGGAGACCACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACCCTGACCAGCCTG
AAGAGCCTGTTGCGCAACGACCCCTGAGCCAAGAATTGAGCCCGTGGACCCCAACCTGGAGCCCTGG
AACCACCCCGGCAGCCAGCCCAAGACCGCGGCAACAAGTGCTACTGCAAGCACTGCAGCTACCACTGC
CTGGTGAGCTTCCAGACCAAGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCGCAGCGCCGAGC
GCCCCCCCCAGCAGCGAGGACCACCAGAACCCCATCAGCAAGCAGCCCCTGCCCCAGACCCGCGCGAC
CCACCGGCAGCGAGGAGAGCAAGAAGAAGGTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGG
GCCGCGCGCAGCGCGACAGCGACGAGGCCCTGCTGCAGGCGGTGCGCATCATCAAGATCCTGTACAG
AGCAACCCCTACCCCAAGCCGAGGGCACCCGCCAGGCCGACCTGAACCGCGCGCGCTGGCGCGCC
CGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATCCTGAGCACCTGCCCTGGGCGCCCCCGCGAGCCC
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AGCATCGTGGGCTGGCCCGCGTGCAGCGAGCGCATCCGCCGACCGAGCCCGCGCGAGGGCGTGGGC
GCCGCCAGCCAGGACCTGGACAAGCACGGCGCCCTGACCAGCAGCAACACCGCCGCAACAACGCGGAC
TGCCTGCTGGTGGAGGCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCAGGTGCCCCCTG
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GGCTGGCAGAACTACACCCCGGCCCGCGTGCCTACCCCTGACCTTCGGCTGGTGTCTCAAGCTG
GTGCCCGTGGACCCCGCGAGGTGGAGGAGGCCAACAAGGGCGAGAACAATGCCTGCTGCACCCCATG
AGCCAGCACGGCATGGAGGACGAGGACCGGAGGTGCTGAAGTGGAAGTTCGACAGCAGCCTGGCCCGC
GCCACATGGCCCGGAGCTGCACCCCGAGTACTACAAGGACTGCGCCTAA

Figure 18
(Sheet 1 of 1)

gp120mod.TV1.del118-210

```
1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggt ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc
361 ggcgcctgcc ccaaggtgag ctctgacccc atccccatcc actactgcgc ccccgccggc
421 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggccccctg ctacaacgtg
481 agcaccgtgc agtgcaccca cggcatcaag cccgtggtga gcaccagct gctgctgaac
541 ggcagcctgg ccgaggaggg catcatcatc cgcagcgaga acctgaccga gaacaccaag
601 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcaccgccc caacaacaac
661 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccacca cgcgtgatc
721 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gaccctgcag
781 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagt caagccccac
841 gccggcgggc acctggagat caccatgcac agcttcaact gccggggcga gttctttac
901 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
961 aacggcaaca gcagcagccc catcaccctg cagtgcaga tcaagcagat cgtgcgcatg
1021 tggcagggcg tgggccaggc cacctacgcc ccccccacg ccggcaacat cacctgccgc
1081 agcaacatca ccggcatcct gctgaccgcg gacggcggt tcaacaccac caacaacacc
1141 gagaccttc gccccggcg cggcgacat gcgcgacaact ggcgcagcga gctgtacaag
1201 tacaaggtgg tggagatcaa gccctgggc atcgcccca ccaaggccaa gcgccgcgtg
1261 gtgcagcgcg agaagcgcta a
```


Figure 19
(Sheet 1 of 1)

gp120mod.TV1.delV1V2

```

1  atgcgcgtga  tgggcaccca  gaagaactgc  cagcagtggg  ggatctgggg  catcctgggc
61  ttctgggatgc  tgatgatctg  caacaccgag  gacctgtggg  tgaccgtgta  ctacggcggtg
121  ccggtgtggc  gcgacgccaa  gaccaccctg  ttctgcgcca  gcgacgccaa  ggccctacgag
181  accgaggtgc  acaacgtgtg  ggccacccac  gcctgcgtgc  ccaccgacce  caacccccag
241  gagatcgtgc  tgggcaacgt  gaccgagaac  ttcaacatgt  ggaagaacga  catggccgac
301  cagatgcacg  aggacgtgat  cagcctgtgg  gaccagagcc  tgaagccctg  cgtgaagctg
361  acccccctgt  gctggggcgc  cggcaactgc  aacaccagca  ccatcaccca  ggccctgcccc
421  aaggtgagct  tcgaccccat  ccccatccac  tactgcgccc  ccgcccggcta  cgccatcctg
481  aagtgaaca  acaagacctt  caacggcacc  ggcccctgct  acaacgtgag  caccgtgcag
541  tgcacccacg  gcatcaagcc  cgtggtgagc  acccagctgc  tgctgaacgg  cagcctggcc
601  gaggagggca  tcatcatccg  cagcgagaac  ctgaccgaga  acaccaagac  catcatcgtg
661  cacctgaacg  agagcgtgga  gatcaactgc  acccgcccca  acaacaacac  ccgcaagagc
721  gtgcgcatcg  gccccggcca  ggcccttctac  gccaccaacg  acgtgatcgg  caacatccgc
781  cagggccact  gcaacatcag  caccgaccgc  tggacaaga  ccctgcagca  ggtgatgaag
841  aagctgggcg  agcacttccc  caacaagacc  atccagttca  agccccacgc  cggcggcgac
901  ctggagatca  ccatgcacag  cttcaactgc  cgccggcgagt  tcttctactg  caacaccagc
961  aacctgttca  acagcaccta  ccacagcaac  aacggcacct  acaagtacaa  cggcaacagc
1021  agcagcccca  tcaccctgca  gtgcaagatc  aagcagatcg  tgcgcatgtg  gcagggcggtg
1081  ggccaggcca  cctacgcccc  ccccatcgcc  ggcaacatca  cctgccgcag  caacatcacc
1141  ggcacacctg  tgacccgcca  cgccggcttc  aacaccacca  acaacaccga  gaccttcgc
1201  cccggcgggc  gcgacatgcg  cgacaactgg  cgcagcgagc  tgtacaagta  caaggtggtg
1261  gagatcaagc  ccctgggcat  cgccccacc  aaggccaagc  gccgcgtggt  gcagcgcgag
1321  aagcgctaa

```

Figure 20
(Sheet 1 of 1)

gp120mod.TV1.delV2

```

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggccctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaacaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgccgg cgccggccgc ctgatcaact gcaacaccag caccatcacc
541 caggcctgcc ccaaggtgag cttcgacccc atccccatcc actactgcgc ccccgccggc
601 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggccccctg ctacaacgtg
661 agcaccgtgc agtgaccca cggcatcaag cccgtggtga gcàcccagct gctgctgaac
721 ggcagcctgg ccgaggaggg catcatcatc cgcagcgaga acctgaccga gaacaccaag
781 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcacccgccc caacaacaac
841 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccacca cgacgtgatc
901 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gaccctgcag
961 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
1021 gccggcgggc acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
1141 aacggcaaca gcagcagccc catcaccctg cagtgcgaaga tcaagcagat cgtgcgcatg
1201 tggcagggcg tgggccaggc cacctacgcc ccccccacg ccggcaacat cacctgcgcg
1261 agcaacatca ccggcatcct gctgacccgc gacggcggt tcaacaccac caacaacacc
1321 gagaccttcc gccccggcgg cggcgacatg cgcgacaact ggcgagcga gctgtacaag
1381 tacaaggtgg tggagatcaa gcccctgggc atcgccccca ccaaggccaa gcgcgcgtg
1441 gtgcagcgcg agaagcgcta a

```

Figure 21
(Sheet 1 of 1)

gp140mod.TV1.del118-210

```

1  atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61  ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caaccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc
361 ggcgctgcc ccaaggtgag cttcgacccc atccccatcc actactgcgc ccccgccggc
421 tacgcatcc tgaagtgcaa caacaagacc ttcaacggca ccggcccctg ctacaacgtg
481 agcaccgtgc agtgaccca cggcatcaag cccgtggtga gcaccagct gctgctgaac
541 ggagcctgg ccgaggagg catcatcatc cgcagcgaga acctgaccga gaacaccaag
601 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcaccgccc caacaacaac
661 acccgcaaga gcgctgcgat cggccccggc caggccttct acgccacca cgacgtgatc
721 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gacctgcag
781 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
841 gccggcgggc acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
901 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
961 aacggcaaca gcagcagccc catcaccctg cagtgaaga tcaagcagat cgtgcgcatg
1021 tggcagggcg tgggccaggc cacctacgcc cccccatcg ccggcaacat cacctgccgc
1081 agcaacatca ccggcatcct gctgaccgcg gacggcggct tcaacaccac caacaacacc
1141 gagaccttc gccccggcgg cggcgacatg cgcgacaact ggcgacgca gctgtacaag
1201 tacaaggtgg tggagatcaa gcccctggg atcgcccca ccaaggccaa gcgccgctg
1261 gtgcagcgcg agaagcgcg cgtgggcata ggcgccgtgt tcctgggctt cctgggcgcc
1321 gccggcagca ccatgggcgc cgccagcatc accctgaccg tgaggcccg ccagctgctg
1381 agcggcatcg tgacgcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
1441 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
1501 tacctgaagg accagcagct gctgggcata tggggctgca gcggccgct gatctgcacc
1561 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
1621 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
1681 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
1741 tggaacaacc tgtggaactg gttcgacatc agcaactggc cctggtacat ctac

```

Figure 22
(Sheet 1 of 1)

gp140mod.TV1.delV1V2

```

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctgggatg tgatgatctg caacaccgag gacctgtggg tgacctgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgggcgc cggcaactgc aacaccagca ccatcaccca ggcctgcccc
421 aagggtgagc tcgaccccat ccccatccac tactgcgccc ccgccggcta cgccatcctg
481 aagtgaaca acaagacctt caacggcacc ggcccctgct acaacgtgag caccgtgcag
541 tgcacccacg gcatcaagcc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc
601 gaggaggcca tcatcatccg cagcgagaac ctgaccgaga acaccaagac catcatcgtg
661 cacctgaacg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc
721 gtgcgcatcg gccccggcca ggccttctac gccaccaacg acgtgatcgg caacatccgc
781 caggcccaact gcaacatcag caccgaccgc tggacaaga ccctgcagca ggtgatgaag
841 aagctgggcg agcacttccc caacaagacc atccagttca agccccacgc cggcggcgac
901 ctggagatca ccatgcacag cttcaactgc cgcggcgagt tcttctactg caacaccagc
961 aacctgttca acagcaccta ccacagcaac aacggcacct acaagtacaa cggcaacagc
1021 agcagcccca tcaccctgca gtgcaagatc aagcagatcg tgcgcatgtg gcagggcgtg
1081 ggccaggcca cctacgcccc ccccatcgcc ggcaacatca cctgccgcag caacatcacc
1141 ggcatacctg tgacccgcga cggcggcttc aacaccacca acaacaccga gaccttccgc
1201 cccggcggcg gcgacatgcg cgacaactgg cgcagcgagc tgtacaagta caaggtgggtg
1261 gagatcaagc ccctgggcat cgccccacc aaggccaagc gccgcgtggg gcagcgcgag
1321 aagcgcgccg tgggcatcgg cgccgtgttc ctgggcttcc tgggcgcgcg cggcagcacc
1381 atgggcgccg ccagcatcac cctgaccgtg caggcccgcc agctgctgag cggcatcgtg
1441 cagcagcaga gcaacctgct gaaggccatc gaggccagc agcacatgct gcagctgacc
1501 gtgtggggca tcaagcagct gcaggccgcg gtgctggcca tcgagcgcta cctgaaggac
1561 cagcagctgc tgggcatctg gggctgcagc ggccgcctga tctgcaccac cgccgtgccc
1621 tggaaacagca gctggagcaa caagagcgag aaggacatct gggacaacat gacctggatg
1681 cagtgggacc gcgagatcag caactacacc ggcctgatct acaacctgct ggaggacagc
1741 cagaaccagc aggagaagaa cgagaaggac ctgctggagc tggacaagtg gaacaacctg
1801 tggaaactggt tcgacatcag caactggccc tggtagatct aa

```

Figure 23
(Sheet 1 of 1)

gp140mod.TV1.delV2

```

1  atgcgcgtga  tgggcaccca  gaagaactgc  cagcagtggg  ggatctgggg  catcctgggg
61  ttctggatgc  tgatgatctg  caacaccgag  gacctgtggg  tgaccgtgta  ctacggcggtg
121 cccgtgtggc  gcgacgccaa  gaccaccctg  ttctgcgcca  gcgacgccaa  ggccctacgag
181 accgaggtgc  acaacgtgtg  ggccaccac  gcctgcgtgc  ccaccgacc  caacccccag
241 gagatcgatg  tgggcaacgt  gaccgagaac  ttcaacatgt  ggaagaacga  catggccgac
301 cagatgcacg  aggacgtgat  cagcctgtgg  gaccagagcc  tgaagccctg  cgtgaagctg
361 accccctgt  gcgtgaccct  gaactgcacc  gacaccaacg  tgaccggcaa  ccgcaccgtg
421 accggcaaca  gcaccaacaa  caccaacggc  accggcatct  acaacatcga  ggagatgaag
481 aactgcagct  tcaacgccgg  cgccggccgc  ctgatcaact  gcaacaccag  caccatcacc
541 caggcctgcc  ccaaggtgag  cttegacccc  atccccatcc  actactgcgc  ccccgccggc
601 tacgccatcc  tgaagtgcga  caacaagacc  ttcaacggca  ccggccctgt  ctacaacgtg
661 agcaccgtgc  agtgcaccca  cgccatcaag  cccgtggtga  gcaccagct  gctgctgaac
721 ggcagcctgg  ccgaggaggg  catcatcatc  cgcagcgaga  acctgaccga  gaacaccaag
781 accatcatcg  tgcacctgaa  cgagagcggt  gagatcaact  gcacccgccc  caacaacaac
841 acccgcaaga  gcgtgcgcat  cggcccccgc  caggccttct  acgccacca  cgacgtgatc
901 ggcaacatcc  gccaggccca  ctgcaacatc  agcaccgacc  gctggaacaa  gaccctgcag
961 caggtgatga  agaagctggg  cgagcacttc  cccaacaaga  ccatccagtt  caagccccac
1021 gccggcgggc  acctggagat  caccatgcac  agcttcaact  gccgcggcga  gttcttctac
1081 tgcaacacca  gcaacctgtt  caacagcacc  taccacagca  acaacggcac  ctacaagtac
1141 aacggcaaca  gcagcagccc  catcaccctg  cagtgcgaaga  tcaagcagat  cgtgcgcatg
1201 tggcagggcg  tgggccaggc  cacctacgcc  cccccatcg  ccggcaacat  caoctgccgc
1261 agcaacatca  ccggcatcct  gctgaccgcg  gacggcggt  tcaacaccac  caacaacacc
1321 gagaccttcc  gccccggcgg  cggcgacatg  cgcgacaact  ggcgagcga  gctgtacaag
1381 tacaaggtgg  tggagatcaa  gcccctgggc  atcgccccca  ccaaggccaa  gcgcgcgtg
1441 gtgcagcgcg  agaagcgcg  cgtgggcatc  ggcgcggtgt  tccctgggctt  cctggggcgc
1501 gccggcagca  ccatgggcgc  cgccagcatc  accctgaccg  tgcaggcccg  ccagctgctg
1561 agcggcatcg  tgcagcagca  gagcaacctg  ctgaaggcca  tcgaggccca  gcagcacatg
1621 ctgcagctga  ccgtgtgggg  catcaagcag  ctgcaggccc  gcgtgctggc  catcgagcgc
1681 tacctgaagg  accagcagct  gctgggcatc  tggggctgca  gcggccgcct  gatctgcacc
1741 accgccgtgc  cctggaacag  cagctggagc  aacaagagcg  agaaggacat  ctgggacaac
1801 atgacctgga  tgcagtggga  ccgcgagatc  agcaactaca  ccggcctgat  ctacaacctg
1861 ctggaggaca  gccagaacca  gcaggagaag  aacgagaagg  acctgctgga  gctggacaag
1921 tggaacaacc  tgtggaactg  gttcgacatc  agcaactggc  cctggtacat  ctaa

```

Figure 24
(Sheet 1 of 1)

gp140mod.TV1.mut7

```

1  atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61  ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgccaa gaccaccctg ttctgcgcca gcgacgccaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gctgcgtgc ccaccgaccc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtagaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaacaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacgccctg
541 ttctaccgcc tggacatcgt gcccctgaac gagaacagcg acaacttcac ctaccgcctg
601 atcaactgca acaccagcac catcaccag gctgccccca aggtgagctt cgaccccatc
661 cccatccact actgcgcccc cgccggctac gccatcctga agtgcaacaa caagaccttc
721 aacggcaccg gcccctgcta caacgtgagc accgtgcagt gacccacgg catcaagccc
781 gtggtgagca ccagctgct gctgaacggc agcctggccg aggaggcat catcatccgc
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtgc acctgaacga gaggctggag
901 atcaactgca cccgcccaa caacaacacc cgcaagagcg tgcgcatcgg ccccgccctg
961 gccttctacg ccaccaacga cgtgatcggc aacatccgcc agggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggcga gcacttcccc
1081 aacaagacca tccagttcaa gcccacgccc ggccggcgacc tggagatcac catgcacagc
1141 ttcaactgcc gcggcgagtt cttctactgc aacaccagca acctgttcaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
1261 tgcaagatca agcagatcgt gcgcatgtgg cagggcggtg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgccgcagc aacatcacgg gcatcctgct gaccgcgac
1381 ggccggttca acaccaccaa caacaccgag accttccgcc ccggcgccgg cgacatgcgc
1441 gacaactggc gcagcgagct gtacaagtac aaggtggtgg agatcaagcc cctgggcatc
1501 gccccacca aggccatcag cagcgtgggt cagagcgaga agagcgccgt gggcatcggc
1561 gccgtgttcc tgggcttcct gggcgccgcc ggacagacca tgggcgccgc cagcatcacc
1621 ctgaccgtgc agggccgcca gctgctgagc ggcacgtgct agcagcagag caacctgctg
1681 aaggccatcg agggccagca gcacatgctg cagctgaccg tgtggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgctac ctgaaggacc agcagctgct gggcatctgg
1801 ggctgcagcg gccgcctgat ctgcaccacc gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caacctgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactggtt cgacatcagc
2041 aactggccct ggtacatcta a

```

Figure 25
(Sheet 1 of 1)

gp140mod.TV1.tpa2

```

1  atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttctgtt
61  tcgcccagca acaccgagga cctgtgggtg accgtgtact acggcgtgcc cgtgtggcgc
121  gacgccaaga ccaccctgtt ctgcgccagc gacgccaagg cctacgagac cgaggtgcac
181  aacgtgtggg ccaccacgc ctgcgtgccc accgacccca accccagga gatcgtgctg
241  ggcaacgtga ccgagaactt caacatgtgg aagaacgaca tggccgacca gatgcacgag
301  gacgtgatca gcctgtggga ccagagcctg aagccctgcg tgaagctgac cccctgtgc
361  gtgacctga actgcaccga caccaacgtg accggcaacc gcaccgtgac cggcaacagc
421  accaacaaca ccaacggcac cggcatctac aacatcgagg agatgaagaa ctgcagcttc
481  aacgccacca ccgagctgcg cgacaagaag cacaaggagt acgccctgtt ctaccgcctg
541  gacatcgtgc cctgaacga gaacagcgac aacttcacct accgcctgat caactgcaac
601  accagcacca tcaccaggc ctgcccgaag gtgagcttcg accccatccc catccactac
661  tgcgcccccg ccggtacgc catcctgaag tgcaacaaca agaccttcaa cggcaccggc
721  ccctgctaca acgtgagcac cgtgcagtgc acccacggca tcaagcccg tgtgagcacc
781  cagctgctgc tgaacggcag cctggccgag gagggcatca tcatccgag cgagaacctg
841  accgaagaaca ccaagaccat catcgtgcac ctgaacgaga gcgtggagat caactgcacc
901  cgcccccaaca acaacacccg caagagcgtg cgcatcggcc ccggccaggc cttctacgcc
961  accaacgacg tgatcggcaa catccgccag gccactgca acatcagcac cgacogctgg
1021  aacaagaccc tgcagcaggt gatgaagaag ctgggcgagc acttcccaa caagaccatc
1081  cagttcaagc ccacgcccgg cggcgacctg gagatcacca tgcacagctt caactgccgc
1141  ggcgagttct tctactgcaa caccagcaac ctgttcaaca gcacctacca cagcaacaac
1201  ggcacctaca agtacaacgg caacagcagc agccccatca ccctgcagtg caagatcaag
1261  cagatcgtgc gcatgtggca gggcgtgggc caggccacct acgccccccc catcgccggc
1321  aacatcacct gccgcagcaa catcacggc atcctgctga cccgcgacgg cggttcaac
1381  accaccaaca acaccgagac cttccgcccc ggcggcgggc acatgcgcga caactggcgc
1441  agcgagctgt acaagtacaa ggtggtggag atcaagcccc tgggcatcgc cccaccaag
1501  gccaaagcgc gctggtgca gcgcgagaag cgcgcctgg gcacggcgc cgtgttctg
1561  ggcttctctg gcgcgcggc cagcaccatg ggcgcgcga gcacacctg gaccgtgcag
1621  gccgcgcagc tgctgagcgg catcgtgcag cagcagagca acctgctgaa ggccatcgag
1681  gccagcagc acatgctgca gctgaccgtg tggggcatca agcagctgca ggccgcgtg
1741  ctggccatcg agcgctacct gaaggaccag cagctgctgg gcatctgggg ctgcagcggc
1801  cgctgatct gcaccaccgc cgtgccctgg aacagcagct ggagcaacaa gagcgagaag
1861  gacatctggg acaacatgac ctggatgcag tgggaccgcg agatcagcaa ctacaccggc
1921  ctgatctaca acctgctgga ggacagccag aaccagcagg agaagaacga gaaggacctg
1981  ctggagctgg acaagtggaa caacctgtgg aactggttcg acatcagcaa ctggccctgg
2041  tacatctaa

```

Figure 26
(Sheet 1 of 1)

gp140.TM.mod.TV1

```

1 atgcgcgatga tgggcaccca gaagaactgc cagcagtggt ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgctg
121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggccctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaaaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacggccctg
541 ttctaccgcc tggacatcgt gcccctgaac gagaacagcg acaacttcac ctaccgcctg
601 atcaactgca acaccagcac catcaccag gcctgcccc aagtgagctt cgaccccatc
661 cccatccact actgcgcccc cgccggctac gccatcctga agtgcaaaa caagaccttc
721 aacggcaccg gcccctgcta caacgtgagc accgtgcagt gcaccacgg catcaagccc
781 gtggtgagca cccagctgct gctgaacggc agcctggccg aggaggcat catcatccgc
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtgc acctgaacga gagcgtggag
901 atcaactgca cccgccccaa caacaacacc cgcaagagcg tgcgcatcgg ccccgccag
961 gccttctacg ccaccaacga cgtgatcgcc aacatccgcc agggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggcca gcacttcccc
1081 aacaagacca tccagttcaa gcccacgcc ggcgccgacc tggagatcac catgcacagc
1141 ttcaactgcc gcggcgagtt cttctactgc aacaccagca acctgttcaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
1261 tgcaagatca agcagatcgt gcgcatgtgg cagggcgtgg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgccgcagc aacatcaccg gcatcctgct gacccgcgac
1381 ggcggttca acaccaaaa caacaccgag accttcgcc ccggcgccgg cgacatgcgc
1441 gacaactggc gcagcgagct gtacaagtac aaggtggtgg agatcaagcc cctgggcatc
1501 gccccacca aggccaagcg ccgcgtggtg cagcgcgaga agcgcgccgt gggcatcggc
1561 gccgtgttcc tgggttccct gggcgccgcc ggcagacca tgggcgccgc cagcatcacc
1621 ctgaccgtgc agggccgcca gctgctgagc ggcacgtgc agcagcagag caacctgctg
1681 aaggccatcg agggccagca gcacatgctg cagctgaccg tgtggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgctac ctgaaggacc agcagctgct gggcatctgg
1801 ggctgcagcg gccgcctgat ctgcaccacc gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caacctgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactggtt cgacatcagc
2041 aactggccct ggtacatcaa gatcttcac atgatcgtgg gcggcctgat cggcctgcgc
2101 atcatcttcg ccgtgctgag catcgtg

```


Figure 27
(Sheet 1 of 1)

gp160mod.TV1.del1118-210

```

1  atgcgctga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61  ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgatc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc
361 gggcctgcc ccaaggtgag ctctgacccc atccccatcc actactgcgc ccccgccggc
421 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggcccctg ctacaacgtg
481 agcaaccgtg agtgcacca cggcatcaag cccgtggtga gcaccagct gctgctgaac
541 ggcagcctgg ccgaggaggg catcatcatc cgcagcgaga acctgaccga gaacaccaag
601 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcaccgccc caacaacaac
661 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccaccaa cgacgtgatc
721 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gacctgcag
781 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
841 gccggcggcg acctggagat caccatgcac agcttcaact gccggggcga gttcttctac
901 tgcacaacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
961 aacggcaaca gcagcagccc catcacctg catgcaaga tcaagcagat cgtgcgcatg
1021 tggcagggcg tgggccaggc cacctacgcc cccccatcg ccggcaacat cactgcccgc
1081 agcaacatca ccggcatcct gctgaccgcg gacggcggct tcaacaccac caacaacacc
1141 gagaccttcc gccccggcgg cggcgacatg cgcgacaact ggcgcagcga gctgtacaag
1201 tacaaggtgg tggagatcaa gcccctgggc atcgccccca ccaaggccaa gcgcgcgctg
1261 gtgcagcgcg agaagcgcgc cgtgggcata ggcgccgtgt tcctgggctt cctgggcgcc
1321 gccggcagca ccattgggcg cgccagcatc acctgaccg tgcaggcccg ccagctgctg
1381 agcggcatcg tgcagcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
1441 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
1501 tacctgaagg accagcagct gctgggcata tggggctgca gcggccgcct gatctgcacc
1561 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
1621 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
1681 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
1741 tggacaacc tgtggaactg gttcgacatc agcaactggc cctggtacat caagatcttc
1801 atcatgatcg tgggcggcct gatcgccctg cgcatactct tcgccgtgct gagcatcgtg
1861 aaccgcgtgc gccagggcta cagccccctg agcttccaga ccctgacccc cagccccgcg
1921 ggcctggacc gcctgggcgg catcgaggag gagggcggcg agcaggaccg cgaccgcagc
1981 atccgcctgg tgagcggcct cctgagcctg gcctgggacg acctgcgcaa cctgtgcctg
2041 ttacagctacc accgcctgcg cgaacttcat ctgatcgccg tgcgcgccgt ggagctgctg
2101 ggccacagca gcctgcgcgg cctgcagcgc ggctgggaga tcctgaagta cctgggcagc
2161 ctggtgcagt actggggcct ggagctgaag aagagcgcca tcagcctgct ggacaccatc
2221 gccatcaccg tggccgaggg caccgaccg atcatcgagc tggtgacgct catctgccgc
2281 gccatcctga acatcccccg ccgcatacgc cagggtctcg aggcgcctt gctgtaa

```

Figure 28
(Sheet 1 of 1)

gp160mod.TV1.delV1V2

```

1  atgcgcgtga  tgggcaccca  gaagaactgc  cagcagtggg  ggatctgggg  catcctgggc
61  ttctgggatgc  tgatgatctg  caacaccgag  gacctgtggg  tgaccgtgta  ctacggcgtg
121  cccgtgtggc  gcgacgcaa  gaccaccctg  ttctgcgcca  gcgacgcaa  ggcctacgag
181  accgaggtgc  acaacgtgtg  ggccaccac  gcctgcgtgc  ccaccgacc  caacccccag
241  gagatcgtgc  tgggcaacgt  gaccgagaac  ttcaacatgt  ggaagaacga  catggccgac
301  cagatgcacg  aggacgtgat  cagcctgtgg  gaccagagcc  tgaagccctg  cgtgaagctg
361  acccccctgt  gcgtgggcgc  cggcaactgc  aacaccagca  ccatcaccca  ggcctgcccc
421  aaggtgagct  tcgaccccat  ccccatccac  tactgcgccc  ccgcccggta  cgccatcctg
481  aagtgaaca  acaagacctt  caacggcacc  ggcccctgct  acaacgtgag  caccgtgcag
541  tgcaccacg  gcatcaagcc  cgtggtgagc  acccagctgc  tgctgaacgg  cagcctggcc
601  gaggaggga  tcacatccg  cagcgagaac  ctgaccgaga  acaccaagac  catcatcgtg
661  cacctgaacg  agagcgtgga  gatcaactgc  acccgcccca  acaacaacac  ccgcaagagc
721  gtgcgcacg  gccccggcca  ggccttctac  gccaccaacg  acgtgatcgg  caacatccgc
781  caggcccact  gcaacatcag  caccgaccgc  tggacaaga  ccctgcagca  ggtgatgaag
841  aagctgggcg  agcacttccc  caacaagacc  atccagttca  agccccacgc  cggcggcgac
901  ctggagatca  ccatgcacag  cttcaactgc  cgcggcgagt  tcttctactg  caacaccagc
961  aacctgttca  acagcaccta  ccacagcaac  aacggcacct  acaagtacaa  cggcaacagc
1021  agcagcccca  tcaccctgca  gtgcaagatc  aagcagatcg  tgcgcatgtg  gcaggggcgtg
1081  ggccaggcca  cctacgcccc  ccccatcgcc  ggcaacatca  cctgcgcgag  caacatcacc
1141  ggcacccctg  tgaccgcgca  cggcggcttc  aacaccacca  acaacaccga  gaccttccgc
1201  cccggcggcg  gcgacatgcg  cgacaactgg  cgcagcgagc  tgtacaagta  caaggtgggtg
1261  gagatcaagc  ccctgggcat  cgccccacc  aaggccaagc  gccgcgtggt  gcagcgcgag
1321  aagcgcgccg  tgggcatcgg  cgccgtgttc  ctgggcttcc  tgggcgcgcg  cggcagcacc
1381  atgggcgccg  ccagcatcac  cctgaccgtg  caggcccgcc  agctgctgag  cggcatcgtg
1441  cagcagcaga  gcaacctgct  gaaggccatc  gaggcccagc  agcacatgct  gcagctgacc
1501  gtgtggggca  tcaagcagct  gcaggccgcg  gtgctggcca  tcgagcgcta  cctgaaggac
1561  cagcagctgc  tgggcatctg  gggctgcagc  ggccgcctga  tctgcaccac  cgccgtgccc
1621  tggaacagca  gctggagcaa  caagagcgag  aaggacatct  gggacaacat  gacctggatg
1681  cagtgggacc  gcgagatcag  caactacacc  ggcctgatct  acaacctgct  ggaggacagc
1741  cagaaccagc  aggagaagaa  cgagaaggac  ctgctggagc  tggacaagtg  gaacaacctg
1801  tggaaactgg  tcgacatcag  caactggccc  tggtagatct  aa

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Figure 29
(Sheet 1 of 1)

gp160mod.TV1.delV2

```

1 atgcgctga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggg
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caaccaccag
241 gagatcgctg tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaaccgtg
421 accggcaaca gcaccaacaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgcccg cgccggccgc ctgatcaact gcaacaccag caccatcacc
541 caggcctgcc ccaaggtgag cttcgacctc atcccatcc actactgcgc ccccgccggc
601 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggcccctg ctacaacgtg
661 agcaccgtgc agtgcaccca cggcatcaag cccgtggtga gcaccagct gctgctgaac
721 ggcagcctgg ccgaggaggg catcatcatc cgcagcgaga acctgaccga gaacaccaag
781 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcaccgccc caacaacaac
841 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccaccaa cgacgtgatc
901 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gaccctgcag
961 caggtgatga agaagctggg cgagcacttc cccaacaaga ccattcagtt caagccccac
1021 gccggcggcg acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
1141 aacggcaaca gcagcagccc catcaccctg cagtgcaga tcaagcagat cgtgcgcatg
1201 tggcagggcg tgggccaggc cacctacgcc ccccccacg ccggcaacat cacctgccgc
1261 agcaacatca ccggcatcct gctgaccgcg gacggcggct tcaacaccac caacaacacc
1321 gagaccttcc gcccggcgcg cggcgacatg cgcgacaact ggcgcagcga gctgtacaag
1381 tacaaggtgg tggagatcaa gcccctggg atcgcccca ccaaggccaa gcgcgcgtg
1441 gtgcagcgcg agaagcgcg cggtgggcat ggcccgctgt tcctgggctt cctgggcgcc
1501 gccggcagca ccatgggcgc cgccagcatc acctgaccg tgcaggcccc ccagctgctg
1561 agcggcatcg tgcagcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
1621 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
1681 tacctgaagg accagcagct gctgggcatc tggggctgca gcggccgcct gatctgcacc
1741 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
1801 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
1921 tggaacaacc tgtggaactg gttcgacatc agcaactggc cctggtacat caagatcttc
1981 atcatgatcg tgggcggcct gatcggcctg cgcacatctc tcgccgtgct gagcatcgtg
2041 aaccgcgtgc gccagggcta cagccccctg agcttccaga ccctgacccc cagccccgcg
2101 ggcctggacc gcctgggcgg catcgaggag gagggcggcg agcaggaccg cgaccgcagc
2161 atccgcctgg tgagcggcct cctgagcctg gcctgggacg acctgcgcaa cctgtgctg
2221 ttcagctacc accgcctgcg cgacttcatc ctgatcgccg tgccgcgcgt ggagctgctg
2281 ggccacagca gcctgcgcgg cctgcagcgc ggctgggaga tcctgaagta cctgggcagc
2341 ctggtgcagt actggggcct ggagctgaag aagagcgcca tcagcctgct ggacaccatc
2401 gccatcaccg tggccgaggg caccgaccgc atcatcgagc tgggtgcagc catctgccgc
2461 gccatcctga acatcccccg ccgcatccgc cagggtctcg aggcgcacct gctgtaa

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Figure 30
(Sheet 1 of 1)

gp160mod.TV1.dv1

```

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtgggt ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcggtg
121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgggcgc cggcaactgc agcttcaacg ccaccaccga gctgcgcgac
421 aagaagcaca aggagtacgc cctgttctac gcctggaca tcgtgcccct gaacgagaac
481 agccacaact tcacctaccg cctgatcaac tgcaacacca gcaccatcac ccaggcctgc
541 cccaaggtga gcttcgacct catccccatc cactactgcg cccccgcgg ctacgccatc
601 ctgaagtgc acaacaagac cttcaacggc accggcccct gctacaacgt gagcaccgtg
661 cagtgcaccc acggcatcaa gcccggtgtg agcaccagc tgctgctgaa cggcagcctg
721 gccgaggagg gcatcatcat ccgcagcgag aacctgaccg agaacaccaa gaccatcatc
781 gtgcacctga acgagagcgt ggagatcaac tgcaccgcg ccaacaacaa caccgcaag
841 agcgtgcgca tcggcccccg ccaggccttc tacgccacca acgacgtgat cggcaacatc
901 cgccaggccc actgcaacat cagcaccgac cgctggaaca agaccctgca gcaggtgatg
961 aagaagctgg gcgagcactt cccaacaag accatccagt tcaagcccca cgccggcggc
1021 gacctggaga tcaccatgca cagcttcaac tgccgcggcg agttcttcta ctgcaacacc
1081 agcaacctgt tcaacagcac ctaccacagc aacaacggca cctacaagta caacggcaac
1141 agcagcagcc ccatcacctc cgagtgaag atcaagcaga tcgtgcgcat gtggcagggc
1201 gtgggccagg ccacctacgc ccccccatc gccggcaaca tcacctgccc cagcaacatc
1261 accggcatcc tegtgaccg cgacggcgtt tcaacacca ccaacaacac cgagaccttc
1321 cgccccggcg gcggcgacat gcgcgacaac tggcgagcgc agctgtacaa gtacaagggtg
1381 gtggagatca agcccctggg catcgcccc accaaggcca agcgccgctg ggtgcagcgc
1441 gagaagcgcg ccgtgggcat cggcgccgtg ttctgggct tcctgggccc cgccggcagc
1501 accatgggcg ccgccagcat caccctgacc gtgcaggccc gccagctgct gagcggcatc
1561 gtgcagcagc agagcaacct gctgaaggcc atcgaggccc agcagcacat gctgcagctg
1621 accgtgtggg gcatcaagca gctgcaggcc cgcgtgctgg ccacgagcg ctacctgaag
1681 gaccagcagc tgctgggcat ctggggctgc agcgccgccc tgatctgcac caccgcccgtg
1741 ccctggaaca gcagctggag caacaagagc gagaaggaca tctgggacaa catgacctgg
1801 atgcagtggg accgcgagat cagcaactac accggcctga tctacaacct gctggaggac
1861 agccagaacc agcaggagaa gaacgagaag gacctgctgg agctggacaa gtggaacaac
1921 ctgtggaact ggttcgacat cagcaactgg ccctggtaca tcaagatctt catcatgatc
1981 gtggcgggcc tgatcgccct gcgcacatc ttccgctgct tgagcatcgt gaaccgctg
2041 cgccagggct acagccccct gagcttccag accctgacct ccagcccccg cggcctggac
2101 cgcttgggcg gcatcgagga ggaggcgggc gagcaggacc gcgaccgcag catccgctg
2161 gtgagcggtt tcctgagcct ggccctgggac gacctgcgca acctgtgctt gttcagctac
2221 caccgcctgc gcgacttcat cctgatcgcc gtgcgcgccc tggagctgct gggccacagc
2281 agcctgcgcg gcctgcagcg cggctgggag atcctgaagt acctgggag cctggtgcag
2341 tactggggcc tggagctgaa gaagagcgcc atcagcctgc tggacaccat cgccatcacc
2401 gtggccgagg gcaccgaccg catcatcgag ctggtgcagc gcatctgccc cgccatcctg
2461 aacatcccc gccgcatccg ccagggcttc gaggccgccc tgctgtaa

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Figure 31 (Sheet 1 of 2)

gp160mod.TV1.dV1-gagmod.BW965

```

1  atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61  ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcttgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 accccctgt gcggtggcgc cggcaactgc agcttcaacg ccaccaccga gctgcgcgac
421 aagaagcaca aggagtacgc cctgttctac cgcctggaca tcgtgccctt gaacgagaac
481 agcgacaact tcacctaccg cctgatcaac tgcaacacca gcaccatcac ccaggcctgc
541 cccaagtgta gcttcgaccc catcccatc cactactgcg ccccgccgg ctacgccatc
601 ctgaagtga acaacaagac cttcaacggc accggccctt gctacaacgt gagcaccgtg
661 cagtgcaccc acggcatcaa gcccgtgttg agcaccacgc tgctgctgaa cggcagcctg
721 gccgaggagg gcatcatcat ccgcagcgag aacctgaccg agaacacca gaccatcatc
781 gtgcacctga acgagagcgt ggagatcaac tgcaccgcgc ccaacaacaa caccgcgaag
841 agcgtgcgca tcggccccgg ccaggccttc tacgccacca acgacgtgat cggcaacatc
901 cgccaggccc actgcaacat cagcaccgac cgctggaaca agaccctgca gcaggtgatg
961 aagaagctgg gcgagcactt ccccaacaag accatccagt tcaagcccca cgcggcggc
1021 gacctggaga tcaccatgca cagcttcaac tgccgcggcg agttcttcta ctgcaacacc
1081 agcaacctgt tcaacagcac ctaccacagc aacaacggca cctacaagta caacggcaac
1141 agcagcagcc ccataccctt gcagtcaag atcaagcaga tcgtgcgcat gtggcagggc
1201 gtgggccagg ccacctacgc ccccccatc gccggcaaca tcacctgccg cagcaacatc
1261 accggcatcc tgctgaccgg cgacggcggc ttcaacacca ccaacaacac cgagaccttc
1321 cgccccggcg gcgcgacat gcgcgacaac tggcgagcg agctgtacaa gtacaaggtg
1381 gtggagatca agccctggg catcgcccc accaaggcca agcgccgctt ggtgcagcgc
1441 gagaagcgcg ccgtgggcat cggcgccgtg ttcttgggct tcttgggcgc cgcggcgagc
1501 accatgggcg ccgccagcat caccctgacc gtgcaggccc gccagctgct gagcggcac
1561 gtgcagcagc agagcaacct gctgaaggcc atcgaggccc agcagcacat gctgcagctg
1621 accgtgtggg gcatcaagca gctgcaggcc cgcgtgctgg ccategagcg ctacctgaag
1681 accagcagc tgctgggcat ctggggctgc agcgccgcgc tgatctgcac caccgcccgtg
1741 ccctgggaaca gcagctggag caacaagcgc gagaaggaca tctgggacaa catgacctgg
1801 atgcagtggg accgcgagat cagcaactac accggcctga tctacaacct gctggaggac
1861 agccagaacc agcaggagaa gaacgagaag gacctgctgg agctggacaa gtggaacaac
1921 ctgtggaact ggttcgacat cagcaactgg ccctgttaca tcaagatctt catcatgatc
1981 gtggcgggcc tgatcgccct ggcacatcat ttccgctgct tgagcatcgt gaaccgctg
2041 cgccagggct acagccccct gagcttccag accctgaccc ccagcccccg cggcctggac
2101 cgcctgggcg gcatcgagga ggaggcggc gagcaggacc gcgaccgag catccgctg
2161 gtgagcggtt tcctgagcct ggctgggac gacctgcgca acctgtgctt gttcagctac
2221 caccgcctgc gcgacttcat cctgatcgcc gtgcgcgccg tggagctgct gggccacagc
2281 agcctgcgcg gcctgcagcg cggctgggag atcctgaagt acctgggcag cctggtgcag
2341 tactggggcc tggagctgaa gaagagcgcc atcagcctgc tggacaccat cgccatcacc
2401 gtggccgagg gcaccgaccg catcatcgag ctggtgcagc gcatctgccg cgccatcctg
2461 aacatcccc cccgcatccg ccagggttc gaggccgccc tgctgtaact cgagcaagtc
2521 tagagggaga ccacaacggg ttccctctag cgggatcaat tccgcccccc cccctaactg
2581 tactggccga agccgcttgg aataaggcgc gtgtgcgttt gtctatatgt tattttccac
2641 catattgcgg tcttttggca atgtgagggc ccggaacctt ggcctgtctt tcttgacgag
2701 cattcctagg ggtctttccc ctctcgccaa aggaatgcaa ggtctgttga atgtcgtgaa
2761 ggaagcagtt cctctggaag cttcttgaag acaacaacg tctgtagcga ccttttgag
2821 gcagcggaac cccccacctg gcgacaggtg cctctgcggc caaaagccac gtgtataaga
2881 tacacctgca aaggcgccac aacccagtg ccacgttgtg agttggatag ttgtggaag
2941 agtcaaatgg ctctcctcaa gcgtattcaa caagggcgct aaggatgcc agaaggtacc
3001 ccattgtatg gcatctgatc tggggcctcg gtgcacatgc tttacatgtg tttagtcgag
3061 gttaaaaaac gtctaggccc cccgaaccac ggggacgtgg ttttcctttg aaaaacagca

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Figure 31
(Sheet 2 of 2)

```

3181 catccgcctg cgccccggcg gcaagaagtg ctacatgatg aaacacctgg tgtggggccag
3241 ccgcgagctg gagaagttcg ccctgaaccc cggcctgctg gagaccagcg agggctgcaa
3301 gcagatcatc cgccagctgc accccgccct gcagaccggc agcgaggagc tgaagagcct
3361 gttcaacacc gtggccaccc tgtactgcgt gcacgagaag atcgaggtcc gcgacaccaa
3421 ggaggccctg gacaagatcg aggaggagca gaacaagtgc cagcagaaga tccagcaggc
3481 cgaggccgccc gacaagggca aggtgagcca gaactacccc atcgtgcaga acctgcaggg
3541 ccagatggtg caccaggcca tcagcccccg caccctgaac gcctgggtga aggtgatcga
3601 ggagaaggcc ttcagccccg aggtgatccc catgttcacc gccctgagcg agggcgccac
3661 cccccaggac ctgaacacga tgttgaacac cgtgggcggc caccaggccc ccatgcagat
3721 gctgaaggac accatcaacg aggaggccgc cgagtgggac cgcgtgcacc ccgtgcacgc
3781 cggccccatc gccccgggcc agatgcgcga gccccgcggc agcgacatcg ccggcaccac
3841 cagcacccctg caggagcaga tcgctgggat gaccagcaac cccccatcc ccgtgggcga
3901 catctacaag cgttggatca tcctgggcct gaacaagatc gtgcggatgt acagccccgt
3961 gagcatcctg gacatcaagc agggcccca ggagcccttc cgcgactacg tggaccgctt
4021 cttcaagacc ctgcgcgccc agcagagcac ccaggaggtg aagaactgga tgaccgacac
4081 cctgctggtg cagaacgcca accccgactg caagaccatc ctgcgcgctc tcggccccgg
4141 cgccagcctg gaggagatga tgaccgcctg ccagggcgtg ggcgggccca gccacaaggc
4201 ccgctgctg gccgaggcga tgagccaggc caacaccagc gtgatgatgc agaagagcaa
4261 cttcaagggc ccccggcgca tcgtcaagtg cttcaactgc ggcaaggagg gccacatcgc
4321 ccgcaactgc cgcgcccccc gcaagaaggg ctgctggaag tgcggcaagg agggccacca
4381 gatgaaggac tgcaccgagc gccaggccaa cttcctgggc aagatctggc ccagccacaa
4441 gggccgcccc ggcaacttc tgagagccg ccccgagccc accgcccccc ccgcccagag
4501 cttccgcttc gaggagacca ccccggccca gaagcaggag agcaaggacc gcgagacctt
4561 gaccagcctg aagagcctgt tcggcaacga cccctgagc caataa

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Figure 32 (Sheet 1 of 2)

gp160mod.TV1.dV1V2-gagmod.BW965

```

1 atgcgcggtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcggtg
121 cccgtgtggc ggcacgcca gaccaccctg ttctgcgcca ggcacgcca ggccctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcggtggcgc cggcaactgc aacaccagca ccatcaccca ggccctgcccc
421 aaggtgagct tcgaccccat ccccatccac tactgcgccc ccgcccggta cgccatcctg
481 aagtgcaca acaagacctt caacggcacc ggccctgtct acaacgtgag caccgtgcag
541 tgcacccacg gcatcaagcc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc
601 gaggagggca tcatcatccg cagcgagaac ctgaccgaga acaccaagac catcatcgtg
661 cacctgaacg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc
721 gtgcgcatcg gccccggcca ggccctctac gccaccaacg acgtgatcgg caacatccgc
781 caggcccaact gcaacatcag caccgaccgc tggaaacaaga ccttcgagca ggtgatgaag
841 aagtgggcg agcacttccc caacaagacc atccagttca agccccacgc cggcgggcagc
901 ctggagatca ccatgcacag cttcaactgc cgcggcgagt tcttctactg caacaccagc
961 aacctgttca acagcaccta ccacgcaaac aacggcacct acaagtacaa cggaacagc
1021 agcagcccca tcaccctgca gtgcaagatc aagcagatcg tgcgcatgtg gcaggggcgtg
1081 ggccaggcca cctacgccc ccccatcgcc ggcaacatca cctgccgcag caacatcacc
1141 ggcatcctgc tgaccgcga cggcggttcc aacaccacca acaacaccga gaccttccgc
1201 cccggcgggc ggcacatgcg cgacaactgg cgcagcgagc tgtacaagta caagtggtg
1261 gagatcaagc cctgggcat cgccccacc aaggccaagc gccgcgtggt gcagcgcgag
1321 aagcgcccg tgggcatcgg cgccgtgttc ctgggttcc tggcgccgc cggcagcacc
1381 atggcgcccg ccagcatcac cctgaccgtg caggcccgcc agctgctgag cggcatcgtg
1441 cagcagcaga gcaacctgct gaaggccatc gaggccagc agcacatgct gcagctgacc
1501 gtgtggggca tcaagcagct gcaggccgc gtgctggcca tcgagcgcta cctgaaggac
1561 cagcagctgc tgggcatctg gggctgcagc ggccgctga tctgcaccac cgccgtgccc
1621 tggaaacagca gctggagcaa caagagcgag aaggacatct gggacaacat gacctggatg
1681 cagtgggacc gcgagatcag caactacacc ggccgtgatc acaacctgct ggaggacagc
1741 cagaaccagc aggagaagaa cgagaagac ctgctggagc tggacaagtg gaacaacctg
1801 tggaaactggt tcgacatcag caactggccc tgggtacatca agatcttcat catgatcgtg
1861 ggccggcctga tcggcctgcg catcatcttc gccgtgctga gcacgtgaa ccgctgccc
1921 cagggttaca gccccctgag cttccagacc ctgaccccca gcccccggg cctggaccgc
1981 ctggcgggca tcgaggagga gggcgcgag caggaccgcg accgcagcat ccgctgggtg
2041 agcggcttcc tgagcctggc ctgggacgac ctgcgcaacc tgtgcctgtt cagctaccac
2101 cgcctgcgcg acttcacct gatcgccgtg cgcgccgtg agctgctggg ccacagcagc
2161 ctgcgcggcc tgcagcgcgg ctgggagatc ctgaagtacc tgggcagcct ggtgcagtac
2221 tggggcctgg agctgaagaa gagcgccatc agcctgctgg acaccatcgc catcaccgtg
2281 gccgagggca ccgaccgcat catcgagctg gtgcagcgca tctgccgcgc catcctgaac
2341 atcccccgcc gcatccgcca gggcttcgag gccgcccctgc tgtaactcga gcaagtctag
2401 agggagacca caacggttcc cctctagcgg gatcaattcc gcccccccc ctaacgttac
2461 tggccgaagc cgcttggaat aaggccgggtg tgcgtttgtc tataatgttat tttccaccat
2521 attgcoctct tttggcaatg tgaggcccg gaaacctggc cctgtcttct tgacgagcat
2581 tcctagggtt ctttccctc tcgccaagg aatgcaagg ctggtgaatg tcgtgaagga
2641 agcagttcct ctggaagctt cttgaagaca aacaacgtct gtagcgacc tttgcaggca
2701 gcggaacccc ccacctggcg acaggtgcct ctgcggccaa aagccacgtg tataagatac
2761 acctgcaaag gcggcacaac ccagtgcca cgttgtagt tggatagttg tggaaagagt
2821 caaatggctc tcctcaagcg tattcaacaa gggcgtgaag gatgcccaga aggtacccca
2881 ttgtatggga tctgatctgg ggcctcggtg cacatgcttt acatgtgttt agtcagggtt
2941 aaaaaacgtc tagggcccc gaaccacggg gacgtggttt tcctttgaaa aacacgataa
3001 taccatgggc gcccgcgcca gcatcctgcg cggcggaag ctggacgcct gggagcgcat
3061 ccgctgcgc cccggcgcca agaagtgcta catgatgaag cacctggtgt gggccagccc

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Figure 32
(Sheet 2 of 2)

```

3181 gatcatccgc cagctgcacc cgcacctgca gaccggcagc gaggagctga agagcctggt
3241 caacaccgtg gccaccctgt actgcgtgca cgagaagatc gagggtccgcg acaccaagga
3301 ggccctggac aagatcgagg aggagcagaa caagtgccag cagaagatcc agcaggccga
3361 ggccgccgac aagggcaagg tgagccagaa ctaccccatc gtgcagaacc tgcagggccga
3421 gatggtgcac caggccatca gccccgcac cctgaacgcc tgggtgaagg tgatcgagga
3481 gaaggccttc agccccgagg tgatccccat gttcaccgcc ctgagcgagg gcgccacccc
3541 ccaggacctg aacacgatgt tgaacaccgt gggcgccac caggccgcca tgcagatgct
3601 gaaggacacc atcaacgagg aggcggccga gtgggaccgc gtgcaccccg tgcacgccgg
3661 ccccatcgcc ccggccaga tgcgcgagcc ccgcggcagc gacatcgccg gcaccaccag
3721 caccctgcag gacagatcg cctggatgac cagcaacccc cccatccccg tgggcgacat
3781 ctacaagcgg tggatcatcc tgggcctgaa caagatcgtg cggatgtaca gcccctgag
3841 catcctggac atcaagcagg gcccgaagga gcccttcgcg gactacgtgg accgcttctt
3901 caagaccctg cgcgccgagc agagcaccca ggaggtgaag aactggatga ccgacacctt
3961 gctggtgcag aacgccaacc ccgactgcaa gaccatcctg cgcgctctcg gcccggcgc
4021 cagcctggag gagatgatga ccgcctgcca gggcgtgggc ggccccagcc acaaggcccg
4081 cgtgctggcc gaggcgatga gccaggccaa caccagcgtg atgatgcaga agagcaactt
4141 caagggcccc cggcgcatcg tcaagtgctt caactgcggc aaggagggcc acatcgcccg
4201 caactgccgc gcccccgca agaagggtg ctggaagtgc ggcaaggagg gccaccagat
4261 gaaggactgc accgagcgcc aggccaactt cctgggcaag atctggccca gccacaaggg
4321 ccgccccggc aacttcctgc agagccgccc cgagcccacc gcccccccg ccgagagctt
4381 ccgcttcgag gagaccaccc ccggccagaa gcaggagagc aaggaccgcg agaccctgac
4441 cagcctgaag agcctgttcg gcaacgaccc cctgagccaa taa

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Figure 33 (Sheet 1 of 2)

gp160mod.TV1.dV2-gagmod.BW965

```

1  atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61  ttctgggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggccctacgag
181 accgaggtgc acaacgtgtg ggccacccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaaaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgccc gcgcggccgc ctgatcaact gcaacaccag caccatcacc
541 caggcctgcc ccaaggtgag cttcgacccc atcccatcc actactgcgc cccgcgggc
601 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggcccctg ctacaacgtg
661 agcaccgtgc agtgcaccca cggcatcaag cccgtgggtg gcaccagct gctgtgaac
721 ggcagcctgg ccgaggagg catcatcatc cgcagcgaga acctgaccga gaacaccaag
781 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcaaccgccc caacaacaac
841 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccaccaa cgacgtgatc
901 ggcacatcc gccaggcca ctgcaacatc agcaccgacc gctggaacaa gaccctgcag
961 caggtgatga agaagctggg cgcgacttcc cccaacaaga ccatccagtt caagcccac
1021 gccggcggcg acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
1141 aacggcaaca gcagcagccc catcacctg cagtgcgaaga tcaagcagat cgtgcgcatg
1201 tggcagggcg tgggccaggc cacctacgcc ccccccctcg ccggcaacat cacctgccgc
1261 agcaacatca ccggcatcct gctgacccgc gacggcggct tcaacaccac caacaacacc
1321 gagaccttcc gcccggcgcc gcgcgacatc cgcgacaact ggcgcagcga gctgtacaag
1381 tacaaggtgg tggagatcaa gcccctgggc atcgccccc ccaaggccaa gcgcgcgtg
1441 gtgcagcgcg agaagcgcg cgtgggcata ggcgcctgt tccctgggctt cctgggcgcc
1501 gccggcagca ccatggcgcc cgcagcatc accctgaccg tgcaggcccc ccagctgctg
1561 agcggcatcg tgcagcagca gagcaacctg ctgaaggcca tcgaggcccc gcagcacatg
1621 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
1681 tacctgaagg accagcagct gctgggcata tggggctgca gcggccgct gatctgcacc
1741 accgcccgtg cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
1801 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
1921 tggaaacaacc tgtggaactg gttcgacatc agcaactggc cctggtacat caagatcttc
1981 atcatgatcg tggggcgccct gatcgccctg cgcacatctc tcgccgtgct gagcatcgtg
2041 aaccgcgtgc gccagggcta cagccccctg agcttccaga cctgacccc cagccccgc
2101 ggcctggacc gcctggcgcc catcgaggag gagggcgccg agcaggaccg cgaccgcagc
2161 atccgcctgg tgagcggctt cctgagcctg gcctgggacg acctgcgcaa cctgtgcctg
2221 ttcagctacc accgcctgcg cgaacttcac ctgatcgccg tgcgcgccgt ggagctgctg
2281 ggccacagca gcctgcgcgg cctgcagcgc ggctgggaga tcctgaagta cctgggcagc
2341 ctggtgcagt actggggcct ggagctgaag aagagcgcca tcagcctgct ggacaccatc
2401 gccatcaccg tggccgaggg caccgaccgc atcatcgagc tggcgcagcg catctgcgc
2461 gccatcctga acatcccccg ccgcacccgc cagggttctg aggccgccct gctgtaactc
2521 gagcaagtct agagggagac cacaacgggt tccctctagc gggatcaatt ccgccccccc
2581 ccctaaccgtt actggccgaa gccgcttgga ataaggccgg tgtgcgtttg tctatatgtt
2641 attttccacc atattgccgt cttttggcaa tgtgagggcc cggaaacctg gccctgtctt
2701 cttgacgagc attcctaggg gtctttcccc tctcgccaaa ggaatgcaag gtctgttgaa
2761 tgtcgtgaag gaagcagttc ctctggaagc ttctgaaga caaacaacgt ctgtagcgac
2821 cttttgcagg cagcgggaacc cccacacctg cgacaggtgc ctctgcggcc aaaagccacg
2881 tgtataagat acacctgcaa aggcggcaca accccagtgc cacgttgtga gttggatagt
2941 tgtggaaaga gtcaaattgg tctcctcaag cgtattcaac aaggggctga aggatgcccc
3001 gaagggtacc cattgtatgg gatctgatct ggggcctcgg tgcacatgct ttcatgtgt
3061 ttagtcgagg ttaaaaaacg tctaggcccc ccgaaccacg gggacgtggg tttccttga
3121 aaaacacgat aataccatgg gcgcccgcgc cagcatcctg cgcggcgcca agctggacgc

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Figure 33
(Sheet 2 of 2)

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3181 ctgggagcgc atccgcctgc gccccggcgg caagaagtgc tacatgatga agcacctggt
3241 gtgggccagc cgcgagctgg agaagttcgc cctgaacccc ggcctgctgg agaccagcga
3301 gggctgcaag cagatcatcc gccagctgca ccccgccctg cagaccggca gcgaggagct
3361 gaagagcctg ttcaacaccg tggccaacct gtactgctg cagagaaga tcgaggtccg
3421 cgacaccaag gaggccctgg acaagatcga ggaggagcag aacaagtgcc agcagaagat
3481 ccagcaggcc gaggccgccc acaagggcaa ggtgagccag aactaccca tcgtgcagaa
3541 cctgcagggc cagatggtgc accaggccat cagccccgc accctgaacg cctgggtgaa
3601 ggtgatcgag gagaaggcct tcagccccga ggtgatcccc atgttcaccg ccctgagcga
3661 gggcgccacc cccaggacc tgaacacgat gttgaacacc gtgggaggcc accaggccgc
3721 catgcagatg ctgaaggaca ccatcaacga ggaggccgcc gagtgggacc gcgtgcaccc
3781 cgtgcacgcc ggccccatcg ccccgggcca gatgcgcgag ccccgggcca gcgacatcgc
3841 cggcaccacc agcacctgc aggagcagat cgcctggatg accagcaacc ccccatccc
3901 cgtgggcgac atctacaagc ggtggatcat cctgggcctg aacaagatcg tgcggatgta
3961 cagccccgtg agcatcctgg acatcaagca gggccccaag gagcccttcc gcgactacgt
4021 ggaccgcttc ttcaagacc tgcgcgccga gcagagcacc caggaggtga agaactggat
4081 gaccgacacc ctgctggtgc agaacgcaa ccccgactgc aagaccatcc tgcgcgtct
4141 cggccccggc gccagcctgg aggagatgat gaccgcctgc cagggcgtgg gcggccccag
4201 ccacaaggcc cgcgtgctgg ccgaggcgat gagccaggcc aacaccagcg tgatgatgca
4261 gaagagcaac ttcaagggcc cccggcgcat cgtcaagtgc ttcaactgcg gcaaggaggg
4321 ccacatcgcc cgcaactgcc gcgcccccg caagaagggc tgctggaagt gcggcaagga
4381 gggccaccag atgaaggact gcaccgagcg ccaggccaac ttctgggca agatctggcc
4441 cagccacaag ggccgccccg gcaacttcct gcagagccgc cccgagcca ccgccccccc
4501 cgccgagagc ttccgcttcg aggagaccac ccccgccag aagcaggaga gcaaggaccg
4561 cgagaccctg accagcctga agagcctgtt cggcaacgac cccctgagcc aataa

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Figure 34
(Sheet 1 of 1)

gp160mod.TV1.tpa2

```

1  atgggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcggt
61  tcgcccagca acaccgagga cctgtgggtg accgtgtact acggcgtgcc cgtgtggcgc
121 gacgccaaga ccacctgtt ctgcgccagc gacgccaagg cctacgagac cgaggtgcac
181 aacgtgtggg ccacccacgc ctgcgtgcc accgaccca accccagga gatcgtgctg
241 ggcaacgtga ccgagaactt caacatgtgg aagaacgaca tggccgacca gatgcacgag
301 gacgtgatca gcctgtggga ccagagcctg aagcctgtgc tgaagctgac cccctgtgc
361 gtgaccctga actgcaccga caccaacgtg accggcaacc gcaccgtgac cggcaacagc
421 accaacaaca ccaacggcac cggcatctac aacatcgagg agatgaagaa ctgcagcttc
481 aacgccacca ccgagctgcg cgacaagaag cacaaggagt acgccctgtt ctaccgcctg
541 gacatcgtgc ccctgaacga gaacagcgac aacttcacct accgcctgat caactgcaac
601 aacagaccca tcaccaggc ctgcccgaag gtgagcttcg accccatccc caaccactac
661 tgcgcccccg ccggctacgc catcctgaag tgcaacaaca agaccttcaa cggcacccgc
721 ccctgtctaca acgtgagcac cgtgcagtgc acccacggca tcaagcccg tggtagcacc
781 cagctgctgc tgaacggcag cctggccgag gagggcacat tcatccgcag cgagaacctg
841 accgagaaca ccaagaccat catcgtgcac ctgaacgaga gcgtggagat caactgcacc
901 cgccccaaca acaacacccg caagagcgtg cgcacgcggc ccggccaggc cttctacgcc
961 accaacgacg tgatcggcaa catccgccag gccactgca acatcagcac cgaccgttgg
1021 aacaagaccc tgcagcaggt gatgaagaag ctgggcgagc acttcccca caagaccatc
1081 cagttcaagc cccacgcggg cggcgacctg gagatcacca tgcacagctt caactgccgc
1141 ggcgagttct tctactgcaa caccagcaac ctgttcaaca gcacctacca cagcaacaac
1201 ggcacctaca agtacaacgg caacagcagc agcccatca ccctgcagtg caagatcaag
1261 cagatcgtgc gcatgtggca gggcgtgggc caggccacct acgccccccc catcgccggc
1321 aacatcacct gccgcagcaa catcacccgc atcctgctga cccgcgacgg cggcttcaac
1381 accaccaaca acaccgagac cttccgcccc ggcggcggcg acatgcgcga caactggcgc
1441 agcgagctgt acaagtacaa ggtggtggag atcaagcccc tgggcatcgc cccaccaag
1501 gccaaagcgc gcgtggtgca gcgcgagaag cgcgcgctgg gcatcggcgc cgtgttctctg
1561 ggcttctctg gcgcgcggcg cagcaccatg ggcgcgcgca gcatcacctt gaccgtgcag
1621 gcccgccagc tgctgagcgg catcgtgcag cagcagagca acctgctgaa ggccatcgag
1681 gcccgagcag acatgctgca gctgaccgtg tggggcatca agcagctgca ggcccgctg
1741 ctggccatcg agcgtacct gaaggaccag cagctgctgg gcatctgggg ctgcagcggc
1801 cgctgatctt gcaccaccgc cgtgccctgg aacagcagct ggagcaacaa gagcgagaag
1861 gacatctggg acaacatgac ctggatgcag tgggaccgcg agatcagcaa ctacaccggc
1921 ctgatctaca acctgctgga ggacagccag aaccagcagg agaagaacga gaaggacctg
1981 ctggagctgg acaagtggaa caacctgtgg aactggttcg acatcagcaa ctggccctgg
2041 tacatcaaga tcttcatcat gatcgtgggc ggctgatcg gcctgcgcat catcttcgcc
2101 gtgctgagca tcgtgaaccg cgtgcgccag ggctacagcc ccctgagctt ccagaccctg
2161 acccccagcc ccccgggcct ggaccgcctg ggcggcatcg aggaggaggg cggcgagcag
2221 gaccgcgacc gcagcatccg cctggtgagc ggcttctctga gcctggcctg ggacgacctg
2281 cgcaacctgt gcctgttcag ctaccaccgc ctgcgcgact tcatcctgat cgccgtgcgc
2341 gccgtggagc tgctgggcca cagcagcctg cgcggcctgc agcgcggctg ggagatcctg
2401 aagtacctgg gcagcctggg gcagtactgg ggcctggagc tgaagaagag cgccatcagc
2461 ctgctggaca ccatcgccat caccgtggcc gagggcaccg accgcatcat cgagctgggtg
2521 cagcgcatct gccgcgcoat cctgaacatc cccgcgcgca tccgccaggg cttcagggcc
2581 gccctgctgt aa

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Figure 35
(Sheet 1 of 2)

gp160mod.TV1-gagmod.BW965

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1 atgcgcggtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctgggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgctgac ccaccgaccc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 accccctgtg cgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaacaa caccaacggc accggcatct acaacatcga ggagatgaag
481 aactgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacgccctg
541 ttctaccgac tggacatcgt gccctgaac gagaacagcg acaacttcac ctaccgcctg
601 atcaactgca acaccagcac catcaccagc gcctgcccc aagtgagctt cgaccccatc
661 cccatccact actgcgcccc cgcgggtac gccatcctga agtgcaacaa caagaccttc
721 aacggcaccg gccctgcta caacgtgagc accgtgcagt gcaccacgg catcaagccc
781 gtggtgagca cccagctgct gctgaacggc agcctggcgg aggaggcat catcatccgc
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtgc acctgaacga gagcgtggag
901 atcaactgca cccgccccaa caacaacacc cgcaagagcg tgcgcatcgg ccccgccag
961 gccttctacg ccaccaacga cgtgatcggc aacatccgcc agggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggcga gcaattcccc
1081 aacaagacca tccagttcaa gcccacggc ggccggcgacc tggagatcac catgcacagc
1141 ttcaactgcc gcggcgagtt cttctactgc aacaccagca acctgttcaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagccccat caccctgcag
1261 tgcaagatca agcagatcgt gcgcatgtgg cagggcggtg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgcccagc aacatcaccg gcatcctgct gaccgcgac
1381 ggcggttca acaccacca caacaccgag acctccgcc ccggcgggcg cgacatgcgc
1441 gacaactggc gcagcgagct gtacaagtac aaggtggtgg agatcaagcc cctgggcatc
1501 gccccacca aggccaagcg ccgctgggtg cagcgcgaga agcgcgccgt gggcatcggc
1561 gccgtgttcc tgggttctct gggcgccgcc ggcaagacca tggcgccgc cagcatcacc
1621 ctgaccgtgc agggccgcca gctgctgagc ggcacgtgac agcagcagag caacctgctg
1681 aaggccatcg agggccagca gcacatgctg cagctgaccg tgtggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgctac ctgaaggacc agcagctgct gggcatctgg
1801 ggctgcagcg gccgctgat ctgcaccacc gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caacctgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactgggt cgacatcagc
2041 aactggccct ggtacatcaa gatcttcac atgatcgtgg cgggctgat cggcctgcgc
2101 atcatcttcg ccgtgctgag catcgtgaac cgcgtgcgcc agggctacag cccctgagc
2161 ttccagaccc tgacccccag ccccgcggc ctggaccgcc tggcgggcat cgaggaggag
2221 ggccggcgagc aggaccgcga ccgcagcatc cgcctggtga gcggcttctt gagcctggcc
2281 tgggacgacc tgcgcaacct gtgcctgttc agctaccacc gcctgcgcga cttcatcctg
2341 atcgccgtgc gcgccgtgga gctgctgggc cacagcagcc tgcgcggcct gcagcgcggc
2401 tgggagatcc tgaagtacct gggcagcctg gtgcagtact ggggctgga gctgaagaag
2461 agcgccatca gcctgctgga caccatcgcc atcaccgtgg ccgagggcac cgaccgcac
2521 atcgagctgg tgcagcgcat ctgcccgcgc atcctgaaca tcccccgcc catccgcccag
2581 ggcttctgag cgcctgctgt gtaactcgag caagtctaga gggagaccac aacgggttcc
2641 ctctagcggg atcaattccg ccccccccc taacgttact ggccgaagcc gcttgggaata
2701 aggcgggtgt gcgtttgtct atatgttatt ttccaccata ttgccgtctt ttggcaatgt
2761 gagggcccg aaacctggcc ctgtcttctt gacgagcatt cctaggggtc tttccctct
2821 cgccaaagga atgcaaggtc tgttgaatgt cgtgaaggaa gcagttcctc tggaaacctc
2881 ttgaagacaa acaacgtctg tagcgacct ttgcaggcag cggaaacccc cacctggcga
2941 caggtgcctc tgcggccaaa agccacgtgt ataagatata cctgcaaagg cggcacacac
3001 ccagtgccac gttgtgagtt ggatagttgt ggaaagagtc aaatggctct cctcaagcgt
3061 attcaacaag gggctgaagg atgccagaa ggtaccccat tgtatgggat ctgatctggg
3121 gcctcggtgc acatgcttta catgtgttta gtcgaggtta aaaaacgtct agggcccccg

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Figure 35
(Sheet 2 of 2)

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3181 aaccacgggg acgtgggttt cctttgaaaa acacgataat accatggggc cccgcgccag
3241 catcctgcgc gccggcaagc tggacgcctg ggagcgcctc cgcctgcgcc cgggcggcaa
3301 gaagtgttac atgatgaagc acctgggtgt ggccagccgc gagctggaga agttcgcctt
3361 gaacccccgc ctgctggaga ccagcgaggg ctgcaagcag atcatccgcc agctgcacc
3421 cgccctgcag accggcagcg aggagctgaa gagcctgttc aacaccgtgg ccaccctgta
3481 ctgcgtgcac gagaagatcg aggtccgcga caccaaggag gccctggaca agatcgagga
3541 ggagcagaac aagtgccagc agaagatcca gcaggccgag gccgccgaca agggcaaggt
3601 gagccagaac taccatcatc tgcagaacct gcagggccag atggtgcacc agggcatcag
3661 cccccgcacc ctgaacgcct gggatgaagg gatcgaggag aaggccttca gccccgaggt
3721 gatcccatg ttcaccgcc tgcgcgaggg cgccacccc caggacctga acacgatgtt
3781 gaacaccgtg ggcggccacc aggcgcccat gcagatgctg aaggacacca tcaacgagga
3841 ggccgccgag tgggaccgcg tgcaccccg gcacgcgggc cccatcgccc cgggccagat
3901 gcgcgagccc cgcggcagcg acatcgccgg caccaccagc accctgcagg agcagatcgc
3961 ctggatgacc agcaaccccc ccatccccgt gggcgacatc tacaagcggg ggatcatcct
4021 gggcctgaac aagatcgtgc ggatgtacag ccccgtagc atcctggaca tcaagcaggg
4081 cccaaggag cccttcgcg actacgtgga ccgcttcttc aagaccctgc gcgccgagca
4141 gagcaccag gaggtgaaga actggatgac cgacacctg ctggtgcaga acgccaaccc
4201 cgactgcaag accatcctgc gcgctctcgg ccccgccgcc agcctggagg agatgatgac
4261 cgctgccag ggcgtgggcg gccccagcca caaggccgc gtgctggccg aggcgatgag
4321 ccaggccaac accagcgtga tgatgcagaa gagcaacttc aagggtcccc ggcgcatcgt
4381 caagtgttc aactgcggca aggagggcca catcgccgc aactgccgcg cccccgcaa
4441 gaagggctgc tggaggtgc gcaaggagg ccaccagatg aaggactgca ccgagcgcca
4501 ggccaacttc ctgggcaaga tctggcccag ccacaagggc cggccgggca acttcctgca
4561 gagccgcccc gagcccaccg cccccccgc cgagagcttc cgcttcgagg agaccacccc
4621 cggccagaag caggagagca aggaccgcga gacctgacc agcctgaaga gcctgttcgg
4681 caacgacccc ctgagccaat aa

```

Figure 36
(Sheet 1 of 1)

int.opt.mut_C (South Africa TV1)

TTCTTGGACGGCATCGACAAGGCCCAGGAGGAGCACGAGCGCTACACAGCAACTGGCGCGCCATGGCC
AACGAGTTCAACCTGCCCCCATCGTGGCCAAGGAGATCGTGGCCAGCGCCGACAAGTGCCAGCTGAAG
GGCGAGGCCATCCACGGCCAGGTGGACTGCAGCCCCGGCATCTGGCAGCTGGCCTGCACCCACCTGGAG
GGCAAGATCATCCTGGTGGCCGTGCACGTGGCCAGCGGCTACATGGAGGCCGAGGTGATCCCCGCCGAG
ACCGGCCAGGAGACCGCCTACTTCATCCTGAAGCTGGCCGGCCGCTGGCCCGTGAAGGTGATCCACACC
GCCAACGGCAGCAACTTCACCAGCACCGCCGTGAAGGCCGCTGCTGGTGGGCCGGCATCCAGCAGGAG
TTCGGCATCCCCTACAACCCCCAGAGCCAGGGCGTGGTGGCGAGCATGAACAAGGAGCTGAAGAAGATC
ATCGGCCAGGTGCGCGACCAGGCCGAGCACCTGAAGACCGCCGTGCAGATGGCCGTGTTTCATCCACAAC
TTCAAGCGCAAGGGCGGCATCGGCGGCTACAGCGCCGGCGAGCGCATCATCGACATCATCGCCACCGAC
ATCCAGACCAAGGAGCTGCAGAAGCAGATCATCCGCATCCAGAACTTCCGCGTGTAACCGCGACAGC
CGCGACCCCATCAAGGGCCCCCGAGCTGCTGTGGAAGGGCGAGGGCGTGGTGGTGTATCGAGGACAAG
GGCGACATCAAGGTGGTGGCCCGCCGCAAGGCCAAGATCATCCGCGACTACGGCAAGCAGATGGCCGGC
GCCGACTGCGTGGCCGGCGGCCAGGACGAGGAC

Figure 37
(Sheet 1 of 1)

int.opt_C (South Africa TV1)

TTCTGGACGGCATCGACAAGGCCAGGAGGAGCACGAGCGCTACACAGCAACTGGCGCGCCATGGCC
AACGAGTTCAACCTGCCCCCATCGTGGCCAAGGAGATCGTGGCCAGCTGCGACAAGTGCCAGCTGAAG
GGCGAGGCCATCCACGGCCAGGTGGACTGCAGCCCCGGCATCTGGCAGCTGGACTGCACCCACCTGGAG
GGCAAGATCATCCTGGTGGCCGTGCACGTGGCCAGCGGCTACATGGAGGCCGAGGTGATCCCCGCCGAG
ACCGGCCAGGAGACCGCCTACTTCATCCTGAAGCTGGCCGGCCGCTGGCCCGTGAAGGTGATCCACACC
GACAACGGCAGCAACTTCACCAGCACCGCCGTGAAGGCCGCTGCTGGTGGGCCGGCATCCAGCAGGAG
TTCGGCATCCCCTACAACCCCGAGGCCAGGGCGTGGTGGAGAGCATGAACAAGGAGCTGAAGAAGATC
ATCGGCCAGGTGCGCGACCAGGCCGAGCACCTGAAGACCGCCGTGCAGATGGCCGTGTTTCATCCACAAC
TTCAAGCGCAAGGGCGGCATCGGCGGCTACAGCGCCGGCGAGCGCATCATCGACATCATCGCCACCGAC
ATCCAGACCAAGGAGCTGCAGAAGCAGATCATCCGCATCCAGAACTTCCGCGTGTAACCGCGACAGC
CGCGACCCCATCTGGAAGGGCCCCGCCGAGTGTGTGGAAGGGCGAGGGCGTGGTGGTGCATCGAGGAC
AAGGGCGACATCAAGGTGGTGGCCCGCGCAAGGCCAAGATCATCCGCGACTACGGCAAGCAGATGGCC
GGCGCCGACTGCGTGGCCGGCGGCCAGGACGAGGAC

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(Sheet 1 of 1)

nef.D106G.-myr19.opt_C (dbl.mutant)

ATGATCCGCCGCACCGAGCCCGCCGCGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCACGGC
GCCCTGACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGCCAGGAGGAGGAG
GAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGTGCCCCTGCGCCCCATGACCTACAAGGCCGCCCTTCGAC
CTGAGCTTCTTCCTGAAGGAGAAGGGCGGCCTGGAGGGCCTGATCTACAGCAAGAAGCGCCAGGAGATC
CTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCCGGCCCCGGC
GTGCGCTACCCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACCCCCGCGAGGTGGAGGAG
GCCAACAAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGC
GAGGTGCTGAAGTGGAAGTTCGACAGCAGCCTGGCCCGCCGCCACATGGCCCGGAGCTGCACCCCGAG
TACTACAAGGACTGCGCC

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Figure 39
(Sheet 1 of 1)

p15RnaseH.opt_C

TACGTGGACGGCGCCGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGA
CCGGGGCCGGCAGAAGATCGTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGC
AGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGC
CAGTACGCCCTGGGCATCATCCAGGCCAGCCCCACAAGAGCGAGAGCGAGCTGGTGAA
CCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCA
CAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGG
TGCTC

Figure 40
(Sheet 1 of 1)

p2Pol.opt.YMWM_C

GCCACCATGCGCCGAGGCCATGAGCCAGGCCACCAGCGCCAACATCCTGATGCAGCGCAGCAACTTCAAG
GGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCGCGCC
CCCCGCAAGAAGGGCTGCTGGAAGTGCAGGCCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAG
GCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAAC
CGCGCCAACAGCCCCACCAGCCGCGAGCTGCAGGTGCGCGGGCACAACCCCCGAGCGAGGCCGGCGCC
GAGCGCCAGGGCACCTGAACTTCCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTG
GGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGGCGCCGACGACACCGTGTGGAGGAGATGAGCCTG
CCCGGCAAGTGGAAGCCCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAG
ATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGCCCCACCCCGTGAACATC
ATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCTTGAACCTTCCCATCAGCCCCATCGAGACCGTG
CCCGTGAAGCTGAAGCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCGTGACCGAGGAGAAGATC
AAGGCCCTTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCCGAGAAC
CCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTC
CGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGCGCCTG
AAGAAGAAGAAGAGCGTGACCGTGTGGACGTGGGCGACGCTTACTTCAGCGTGCCCCGTGGACGAGGAC
TTCGCGAAGTACACCGCCTTCAACATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTAC
AACGTGCTGCCCCAGGGCTGGAAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAG
CCCTTCCGCGCCCGCAACCCGAGATCGTGATCTACCAGGCCCCCCGTGTACGTGGGCGAGCAGCTGGAG
ATCGGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCC
GACAAGAAGCACCAAGAGAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAG
CCCATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAAC
TGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCGCCAAGGCC
CTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACC GCGAGATCCTGCGC
GAGCCCCTGCACGGCGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCAC
GACCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATG
CGCACCGCCCAACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATC
GTGATCTGGGGCAAGACCCCCAAGTTCCGCTGCCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACC
GACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCTGGTGAAGCTGTGG
TACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCGCCCAACCGCGAG
ACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCGGCGAGAAGATCGTGAGCCTGACCGAGACC
ACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATC
GTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCGACAAGAGCGAGAGCGAGCTGGTG
AACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCACAAGGGC
ATCGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTGTTCCTGGACGGC
ATCGATGGCGGCATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATC
GATTAAAAGCTTCCCGGGCTAGCACCGGT

Figure 41
(Sheet 1 of 1)

p2Polopt.YM_C

GTTCGACGCCACCATGGCCGAGGCCATGAGCCAGGCCACCAGCGCCAACATCCTGATGCAGCGCAGCAAC
TTCAAGGGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCCCAACTGC
CGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAG
CGCCAGGCCAACTTCTTCCGCGAGGACCTGGCCCTCCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAG
CAGAACC CGCCCAACAGCCCCACCAGCCGCGAGCTGCAGGTGCGCGGCGACAACCCCCGACGCGAGGGCC
GGCGCCGAGCGCCAGGGCACCTGAACTTCCCCCAGATCACCCCTGTGGCAGCGCCCCCTGGTGAGCATC
AAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGCGCCGACGACACCGTGCTGGAGGAGATG
AGCCTGCCCCGGCAAGTGGAAGCCCAAGATGATCGGCGGCATCGGCGGCTTTCATCAAGGTGCGCCAGTAC
GACCAGATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCCGTG
AACATCATCGGCGCAACATGCTGACCCAGCTGGGCTGCACCCCTGAACTTCCCCATCAGCCCCATCGAG
ACCGTGCCCCGTGAAGCTGAAGCCCCGGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCTGACCGAGGAG
AAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCC
GAGAACCCTTACAACACCCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTG
GACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCCGCC
GGCCTGAAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTACTTCAGCGTGCCCTGGAC
GAGGACTTCCGCAAGTACACCGCCTTACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTAC
CAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATC
CTGGAGCCCTTCCGCGCCCGCAACCCCCGAGATCGTGATCTACCAGGCCCCCTGTACGTGGGCAGCGAC
CTGGAGATCGGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACC
ACCCCCGACAAGAAGCACCAAGAGAGCCCCCTTCTGTGGATGGGCTACGAGCTGCACCCCCGACAAG
TGGACCGTGACGCCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTG
GGCAAGCTGAACTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGC
GGCGCCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGC
GAGATCCTGCGCGAGCCCCGTGCACGGCTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAG
AAGCAGGGCCACGACCAAGTGACCTACCAGATCTACCAGGAGCCCCTTCAAGAACCTGAAGACCGGCAAG
TACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCC
ATGGAGAGCATCGTGATCTGGGGCAAGACCCCCAAGTTCCGCTGCCATCCAGAAGGAGACCTGGGAG
ACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCCCCCTG
GTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCC
GCCAACC CGGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGCCCGCAGAAGATCGTGAGC
CTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGC
GAGGTGAACATCGTGACCGACAGCCAGTACGCCCCGGGCATCATCCAGGCCAGCCCCGACAAGAGCGAG
AGCGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCC
GCCCACAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTG
TTCTTGACGGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGC
GGCCCTAGGATCGATTAAAGCTTCCCGGGGCTAGCACCGGT

Figure 42
(Sheet 1 of 1)

p2Polopt_C

GCCACCATTGGCCGAGGCCATGAGCCAGGCCACCAGCGCCAACATCCTGATGCGAGCGCAGCAACTTCAAG
GGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCCGAACTGCCGCGCC
CCCCGCAAGAAGGGCTGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAG
GCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAAC
CGCGCCAACAGCCCCACCAGCCGCGAGCTGCAGGTGCGCGGCGACAACCCCCGAGCGAGGCCGCGCGCC
GAGCGCCAGGGCACCCCTGAACCTTCCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTG
GGCGGCCAGATCAAGGAGGCCCTGCTGGACACCGGCGCGGACGACACCGTGCTGGAGGAGATGAGCCTG
CCCCGCAAGTGGAAGCCCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAG
ATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCGTGAACATC
ATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCCCTGAACCTTCCCCATCAGCCCCATCGAGACCGTG
CCCGTGAAGCTGAAGCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATC
AAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCCGAGAAC
CCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTC
CGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGCGCCTG
AAGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTTACTTCAGCGTGCCCCCTGGACGAGGAC
TTCCGCAAGTACACCGCCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAATAC
AACGCTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCAGAGCAGCATGACCAAGATCCTGGAG
CCCTTCCGCGCCCGCAACCCGAGATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCGAGCGAC
CTGGAGATCGGCCAGCACCGGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACC
ACCCCCGACAAGAAGCACCAAGGAGCCCCCTTCCTGTGGATGGGCTACGAGCTGCACCCCGACAAG
TGGACCGTGCAGCCCATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTG
GGCAAGCTGAACCTGGGCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGC
GGCGCCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGC
GAGATCCTGCGCGAGCCCGTGACGGCGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAG
AAGCAGGGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAG
TACGCCAAGATGCGCACCGGCCACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCC
ATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCTGCCCATCCAGAAGGAGACCTGGGAG
ACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCTTG
GTGAAGCTGTGGTACCAAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCC
GCCAACC GCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGCCGGCAGAAGATCGTGAGC
CTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCGAGC
GAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCAGGCCAGCCGACAAGAGCGAG
AGCGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCC
GCCCAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTG
TTCTTGGACGGCATCGATGGCGGCATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCGAGCGG
GGCCCTAGGATCGATTAAAGCTTCCCGGGGCTAGCACCGGT

Figure 43
(Sheet 1 of 1)

p2PolTatRevNef.opt_c

GTCTGACGCCACCATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCACCCCGGCAGCCAGCCCAAG
ACCGCCGGCAACAAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGC
CTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCAGCAGCGAGGACCAC
CAGAACCCCATCAGCAAGCAGCCCTGCCCCAGACCCGCGCGACCCACCGGCAGGAGAGCAAG
AAGAAGGTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGCCGGCCGAGCGCGACAGCGAC
GAGGCCCTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAG
GGCACCCCGCCAGGCCGACCTGAACCGCCGCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATC
AGCGAGCGCATCCTGAGCACCTGCCCTGGGCCGCCCGCCGAGCCCGTGCCCTTCCAGCTGCCCCCGAC
CTGCCCTGCACATCGACTGCAGCGAGAGCAGCGGCACACGCGCACCCAGCAGAGCCAGGGCACCACC
GAGGGCGTGGGCAGCCCCCTCGAGGCCGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGTG
CGCGAGCGCATCCGCCGACCGAGCCCGCCGCGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAG
CACGGCGCCCTGACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGCCAGGAG
GAGGAGGAGGAGTGGGCTTCCCGTGCGCCCCAGGTGCCCTGCGCCCCATGACCTACAAGGCCGCC
TTGACCTGAGCTTCTTCTGAAGGAGAAGGGCGGCCCTGGAGGGCTGATCTACAGCAAGAAGCGCCAG
GAGATCCTGGACCTGTGGGTGTACCAACCCAGGGCTTCTTCCCGGCTGGCAGAACTACACCCCGGC
CCCGCGTGGCTACCCCTGACCTTCGGCTGGTGTCTCAAGCTGGTGCCTGGACCCCGCGAGGTG
GAGGAGGCCAACAGGGCGAGAACAATGCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAG
GACCGCGAGGTGCTGAAGTGAAGTTCGACAGCAGCCTGGCCCGCCGCCACATGGCCCGGAGCTGCAC
CCCGAGTACTACAAGGACTGCGAATTCGCCGAGGCCATGAGCCAGGCCACAGCGCCAACATCCTGATG
CAGCGCAGCAACTTCAAGGGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATC
GCCCCGAACTGCCGCGCCCCCGCAAGAAGGGCTGTGGAAAGTGGCGCAAGGAGGGCCACCATGAAG
GACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTGGCCTTCCCCAGGGCAAGGCCCGCGAG
TTCCCCAGCGAGCAGAACCGCGCCAACAGCCCCACAGCCGCGAGCTGCAGGTGCGCGGCGACAACCCC
CGCAGCGAGGCCGCGCGCCGAGCGCCAGGGCACCTGAACTTCCCCAGATCACCTGTGGCAGCGCCCC
CTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGGCCCGCAGCACCCGTG
CTGGAGGAGATGAGCCTGCCCGCAAGTGAAGCCCAAGATGATCGCGGCATCGGCGGCTTCATCAAG
GTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGC
CCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCTGAACCTTCCCCATC
AGCCCCATCGAGACCGTGCCTGTAAGCTGAAGCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCC
CTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACC
AAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCAACCAAGTGG
CGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATC
CCCCACCCCGCGCCTGAAGAAGAAGAGCGTGACCGTGTGAGCGTGGGCAGCGCTACTTCAGC
GTGCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTACCATCCCCAGCATCAACAACGAGACCCCC
GGCATCCGCTACAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGC
ATGACCAAGATCCTGGAGCCCTTCCGCGCCGCAACCCGAGATCGTGATCTACCAGGCCCCCTGTAC
GTGGGAGCGACCTGGAGATCGGCCAGCACCGGCCAAGATCGAGGAGCTGCGCAAGCACCTGTGCGC
TGGGGCTTACCACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGCCCCATCGAGCTGCACCCC
GACAAGTGGACCGTGCAGCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAG
CTGGTGGGCAAGCTGAACCTGGGCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTG
CTGCGCGGCGCCAAGGCCCTGACCGACATCGTCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAG
AACC CGAGATCTGCGCGAGCCCGTGACGCGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAG
ATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACC
GGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAG
ATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCCTGCCATCCAGAAGGAGACC
TGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTGAACACCCCC
CCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCGAGACCTTCTACGTGGAC
GGCGCCGCCAACC CGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCCGCGAGAAGATC
GTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGC
GGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCGACAAG
AGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACTGTAGCTGG
GTGCCCGCCACAAGGGCATCGGCGCAACGAGCAGATCGACAAGCTGGTGGCAAGGGCATCCGCAAG
GTGCTGTAA

Figure 44
(Sheet 1 of 1)

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GCCACCATGGCCGAGGCCATGAGCCAGGCCACCAGCGCCAACATCCTGATGCAGCGCAGCAACTTCAAG
GGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCCGCAACTGCCGCGCC
CCCCGCAAGAAGGGCTGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCCGAGCGCCAG
GCCAACTTCTTCCGCGAGGACCTGGCCCTTCCCCCAGGGCAAGGCCCCGCGAGTTCCTCCAGCGAGCAGAAC
CGCGCCAACAGCCCCACCAGCCGCGAGCTGCAGGTGCGCGGCGACAACCCCGCAGCGAGGCCGCGGCC
GAGCGCCAGGGCACCTGAACCTTCCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTG
GGCGGCCAGATCAAGGAGGCCCTGCTGGACACCGGCGCCGACGACACCGTGTGGAGGAGATGAGCCCTG
CCCCGCAAGTGAAGCCCAAGATGATCGGCGGCATCGCGGGCTTCATCAAGGTGCGCCAGTACGACCAG
ATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGCCCCACCCCGTGAACATC
ATCGGCCGCAACATGCTGACCCAGCTGGGCTGCACCCCTGAACCTTCCCCATCAGCCCCATCGAGACCGTG
CCCCGTAAGCTGAAGCCCGGCATGGACCGGCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATC
AAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCGGAGAAC
CCCTACAACACCCCCGTGTTCCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAGCTGGTGGACCTTC
CGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACC CGCGGCCCTG
AAGAAGAAGAAGCGTGACCGTGTGGACGTGGGCGACGCCCTACTTCAGCGTGCCCTGGACGAGGAC
TTCCGCAAGTACACCGCCTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCGATAC
AACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAG
CCCTTCCGCGCCCGCAACCCCGAGATCGTGATCTACCGATACATGGACGACCTGTACGTGGGCGAGCGAC
CTGGAGATCGGCCAGCACCGCGCCAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACC
ACCCCGCAAGAAGCACCAAGAAGGAGCCCCCTTCTGTGGATGGGCTACGAGCTGCACCCCGACAAG
TGGACCGTGCAGCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTG
GGCAAGCTGAACCTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGC
GGCGCCAAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCGAGCTGGAGCTGGCCGAGAACC GC
GAGATCTGCGCGAGCCCCGTGCACGGCGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAG
AAGCAGGGCCACGACAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAG
TAGGCCAAGATGCGCACCGCCACACCAACGACCTGAAGCAGCTGACCGAGGCCGTGCAGAGATCGCC
ATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCTGCCCATCCAGAAGGAGACCTGGGAG
ACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTAACACCCCGCCCTG
GTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGCGCGCCGAGACCTTCTACGTGGACGGCGCC
GCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCGGCGAGAAGATCGTGAGC
CTGACCGAGACCAACCAAGAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGAGC
GAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCGACAAGAGCGAG
AGCGAGCTGGTGAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCC
GCCCAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAAGCAAGGGCATCCGCAAGGTGCTG
GAATTCGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACACCCCGGCAGCCAGCCCAAGACCGCCTGC
AACAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGTGCTTCCAGACCAAGGGCCTGGGCATC
AGCTACGGCCGCAAGAAGCGCCGCGCAGCGCCGACGCCCCCCCCAGCAGCGAGGACCACCAGAACCCC
ATCAGCAAGCAGCCCTGCCCGAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAGGTG
GAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGCGCGCCGACGCGGCGACGCGAGGCGCCCTG
CTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACCCGC
CAGGCCCGCAAGAACC GCGCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGC
ATCCTGAGCACCTGCCTGGGCCGCCCCGCGAGCCCGTGCCTTCCAGCTGCCCCCATCGAGCGCCTG
CACATCGACTGCAGCGAGAGCAGCGGCACCGCGCACCCAGCAGAGCCAGGGCACACCAGAGGGCGTG
GGCAGCCCCCTCGAGGGCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGTGCGCGAGCGC
ATCCGCGCGACCGAGCCCGCCGCGAGGGCGCCGCGAGGGCGCCGCGAGGGCGTGGGCGCCGCGCAGC
CAGGACCTGGACAAGCACGCGCGCCCTGACCAGCAGCAACACCGCCGCAACAACGCCGACTGCGCCCTGG
CTGGAGGCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCAGGTGCCCTGCGCCCCATG
ACCTACAAGGCCGCTTCGACCTGAGCTTCTTCTTGAAGGAGAAGGGCGGCTGGAGGGCTGATCTAC
AGCAAGAAGCGCCAGGAGATCTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGACTGGCAG
AATACACCCCGGCCCCGCGCTGCGCTACCCCTGACCTTCCGCTGGTGTCTCAAGCTGGTGGCCGTG
GACCCCGCGAGGTGGAGGAGGCCAACAAGGGCGAGAACAACCTGCTGACCCCATGAGCCAGCAC
GGCATGGAGGACGAGGACCGCGAGGTGCTGAAGTGAAGTTGACAGCAGCCTGGCCCGCGCCACATG
GCCCCGAGCTGCACCCGAGTACTACAAGGACTGC

Figure 45
(Sheet 1 of 1)

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GTGACGCCACCATGGCCGAGGCCATGAGCCAGGCCACCAGCGCCAACATCCTGATGCAGCGCAGCAAC
TTCAAGGGCCCCAAGCGCATCATCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGC
CGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCAG
CGCCAGGCCAACTTCTTCGCGAGGACCTGGCCTTCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAG
CAGAACCCGCGCAACAGCCCCACCAGCCGCGAGCTGCAGGTGCGCGGCGACAACCCCCGACGCGAGGCC
GGCGCCGAGCGCCAGGGCACCTGAACTTCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATC
AAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGGCGCGACGACACCGTGTGGAGGAGATG
AGCCTGCCCCGCAAGTGGAAGCCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTAC
GACCAGATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGCCCCACCCCGTG
AACATCATCGGCCCAACATGCTGACCCAGCTGGGCTGCACCTGAACTTCCCCATCAGCCCCATCGAG
ACCGTGCCCGTGAAGCTGAAGCCCGGCATGGACGGCCCCAAGGTGAAGCAGTGCCCTGACCGAGGAG
AAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCC
GAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTG
GACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCC
GGCCTGAAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCTACTTCAGCGTGCCCTGGAC
GAGGACTTCCGCAAGTACACCGCTTACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTAC
CAGTACAACGTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGATGACCAAGATC
CTGGAGCCCTTCCGCGCCCGCAACCCCGAGATCGTGATCTACCAGGCCCCCTGTACGTGGGCGAGCGAC
CTGGAGATCGGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGTGGGGCTTCACC
ACCCCGGACAAGAAGCACCAGAAGGAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACC
GTGCAGCCCATCGAGCTGCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAG
CTGAAGTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGCGCC
AAGGCCCTGACCGACATCGTGCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATC
CTGCGCGAGCCCGTGACGGCGTGCTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAG
GGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCC
AAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAG
AGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCTGCCCATCCAGAAGGAGACCTGGGAGACCTGG
TGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTAACACCCCCCTGGTGAAG
CTGTGGTACCGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAAC
CGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGCGCGGCGAGAAGATCGTGAGCCTGACC
GAGACCAACCAAGACAGACAGCTGACGCCCCGGGCATCATCCAGGCCAGCCGACAGAAGAGCGAGCGAG
AACATCGTGACCGACAGCCAGTACGCCCCGGGCATCATCCAGGCCAGCCGACAGAAGAGCGAGCGAG
CTGGTGAACAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCAC
AAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTGGAATTC
GAGCCCGTGGACCCCAACCTGGAGCCCTGGAACACCCCGGCGAGCCAGCCCAAGACCGCCGCGCAACAG
TGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGCTTGGGCATCAGCTAC
GGCCGCAAGAAGCGCCGCCAGCGCCGACGCCCCCCCCAGCAGCGAGGACCAACAGAACCCCATCAGC
AAGCAGCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGCAAGAAGAAGGTGGAGAGC
AAGACCGAGACCGACCCCTTCGACCCCGGGCGCGGCGCAGCGGCGACAGCGACGAGGCCCTGCTGCAG
GCCGTGCGCATCATCAAGATCCTGTACCAAGCAACCCCTACCCCAAGCCCGAGGGCACCCGCCAGGCC
GACCTGAACCGCCCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATCCTG
AGCACCTGCCTGGGCCGCCCGCCGAGCCCTGCCCCCTTCAGCTGCCCCCGACCTGCGCCTGCACATC
GACTGCAGCGAGAGCAGCGGCACCCAGCGGCACCCAGCAGAGCCAGGGCACCAACCGAGGGCGTGGGCAGC
CCCCTCGAGGCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGCTGCGCGAGCGCATCCGC
CGCACCGAGCCCGCCCGGAGGGCGTGGGCGCCCGCAGCCAGGACCTGGACAAGCAGCGCGCCCTGACC
AGCAGCAACACCGCCGCCAACACGCGGACTGCGCTGGCTGGAGGGCCAGGAGGAGGAGGAGGAGGTG
GGCTTCCCGTGCGCCCCAGGTGCCCTGCGCCCATGACCTACAAGGCCGCTTCGACCTGAGCTTC
TTCTGAAGGAGAAGGGCGGCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATCCTGACCTG
TGGGTGTACCACACCCAGGGCTTCTTCCCGGCTGGCAGAACTACACCCCGGCCCGCGCTGCGCTAC
CCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCTGGACCCCGCGAGGTGGAGGAGGCCAACAAAG
GGCGAGAACAACCTGCTGTCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGCGAGGTGCTG
AAGTGAAGTTTCGACAGCAGCTGGCCCGCCACATGGCCCGGAGCTGCACCCCGAGTACTACAAG
GACTGCGCCTAAATCTAGA

Figure 46
(Sheet 1 of 1)

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CCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTG
CTGGCCACCGCGCGCGACGACACCGTGCTGGAGGAGATGAGCCTGCCCCGCAAGTGAAGCCCAAGATG
ATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAG
AAGGCCATCGGCACCGTGCTGATCGGCCCAACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAG
CTGGGCTGCACCTGAACCTCCCCATCAGCCCCATCGAGACCGTGCCCGTGAAGCTGAAGCCCGCATG
GACGGCCCCAAGGTGAAGCAGTGGCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAG
GAGATGGAGAAGGAGGGCAAGATCACCAAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCC
ATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAG
GACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGCGTGACCGTG
CTGGACGTGGGGGACGCTTACTTCAGCGTGCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTCACC
ATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAAGTACAACGCTGCTGCCCCAGGGCTGGAAG
GGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCGAG
ATCGTGATCTACCAGCCCCCTGTACGTGGGCGAGCGACCTGGAGATCGGCCAGCACCCGCGCAAGATC
GAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACCACCCCGACAAGAAGCACCAGAAGGAGCCC
CCCTTCCTGTGGATGGGCTACGAGCTGCACCCCGACAAGTGGACCGTGACGCCATCGAGCTGCCCGAG
AAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACCTGGGCCAGCCAGATCTAC
CCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCCAAGGCCCTGACCGACATCGTGCCC
CTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGCGCGAGCCCGTGACCGCGCTG
TACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAG
ATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGCAAGTACGCCAAGATGCGCACCGCCACACCAAC
GACGTGAAGCAGCTGACCGAGGCCGTGCGAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACC
CCCAAGTTCCGCTGCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACC
TGGATCCCCGAGTGGGAGTTCTGTGAACACCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAG
CCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCGCCCAACCGCGAGACCAAGATCGGCAAGGCC
GGTACGTGACCGACCGGGGCCGCGAGAAGATCGTGAGCCTGACCGAGACCACCAACAGAAGACCGAG
CTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGAGCGAGGTGAACATCGTGACCGACAGCCAGTAC
GCCCTGGGCATCATCCAGGCCAGCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAG
CTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGCGGCCAAGCAGCAG
ATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTC

Figure 47
(Sheet 1 of 1)

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CCCCAGATCACCCCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTG
CTGGCCACCGCGCCGACGACACCGTGCTGGAGGAGATGAGCCTGCCCCGCAAGTGAAGCCCAAGATG
ATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAG
AAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAG
CTGGGCTGCACCCCTGAACTTCCCCATCAGCCCCATCGAGACCGTGCCCGTGAAGCTGAAGCCCGGCATG
GACGGCCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAG
GAGATGGAGAAGGAGGCAAGATCACCAAGATCGGCCCCGAGAACCCTACAACACCCCGTGTTCGCC
ATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAG
GACTTCTGGGAGGTGACGCTGGGCATCCCCACCCCGCGGCCTGAAGAAGAAGAAGAGCGTGACCGTG
CTGGACGTGGGCGACGCTTACTTACGCGTGCCCTGGACGAGGACTTCCGCAAGTACACCGCCTTACC
ATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAAGTACAACGTGCTGCCCCAGGGCTGGAAG
GGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCCGAG
ATCGTGATCTACCAGGCCCCCTGTACGTGGGCGAGCAGCTGGAGATCGGCCAGCACCAGCGCCAAGATC
GAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTACCACCCCGACAAGAAGCACCAGAAGGAGCCC
CCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGACGCCCATCGAGCTGCCCCGAGAAGGAG
AGCTGGACCGTGAACGACATCCAGAAGCTGGTGGCAAGCTGAAGTGGGCCAGCCAGATCTACCCCGGC
ATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCAAGGCCCTGACCGACATCGTGCCCTGACC
GAGGAGGCCGAGCTGGAGCTGGCCGAGAACCAGCAGATCCTGCGCGAGCCCGTGACGCGCGTACTAC
GACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAGTGGACCTACCAGATCTAC
CAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTG
AAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAG
TTCCGCTGCCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATC
CCCAGTGGGAGTTCTGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATC
ATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTAC
GTGACCGACCGGGGCCGCGAGAAGATCGTGAGCCTGACCGAGACCACCAACAGAAGACCGAGCTGCAG
GCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTG
GGCATCATCCAGGCCAGCCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAGCAGCTGATC
AAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCACAAGGGCATCGGCGGCAACGAGCAGATCGAC
AAGCTGGTGAGCAAGGCATCCGCAAGGTGCTC

Figure 48
(Sheet 1 of 1)

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GCCACCATGCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAG
GAGGCCCTGCTGGACACCGGCGCCGACGACACCGTGTGGAGGAGATGAGCCTGCCCGGCAAGTGAAG
CCCAAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATC
TGCGGCAAGAAGGCCATCGGCACCGTGTGATCGGCCCCACCCCCGTGAACATCATCGGCCGCAACATG
CTGACCCAGCTGGGCTGCACCTGAACCTTCCCCATCAGCCCCATCGAGACCGTGGCCGTGAAGCTGAAG
CCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCC
ATCTGCGAGGAGATGGAGAAGGAGGGCAAGATCACCAGATCGGCCCGGAGAACCCCTACAACACCCCC
GTGTTCCGCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAG
CGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGC
GTGACCGTGTGGACGTGGGCGACGCCCTACTTCAGCGTGGCCCTGGACGAGGACTTCCGCAAGTACACC
GCCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGTGCCCCAG
GGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGC
AACCCCGAGATCGTGATCTACCAGGCCCCCTGTACGTGGGCGAGCAGCTGGAGATCGGCCAGCACCGC
GCCAAGATCGAGGAGCTGCGCAAGCACCCTGCTGCGCTGGGGCTTCACACCCCGGACAAGAAGCACCAG
AAGGAGCCCCCTTCTGCCATCGAGCTGCACCCGACAAGTGGACCGTGCAGCCATCGAGCTGCC
GAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGCAAGCTGAACCTGGGCCAGCCAGATC
TACCCCGGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCCAAGGCCCTGACCGACATCGTG
CCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACC CGGAGATCTGCGCGAGCCCGTGCACGGC
GTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACAGTGGACCTAC
CAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACC
AACGACGTGAAGCAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAG
ACCCCCAAGTTCCGCTGCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCC
ACCTGGATCCCCGAGTGGGAGTTCGTGAACACCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAG
GAGCCCATCATCGCGCCGAGACCTTCTACGTGGACGCGCGCCCAACCGGAGACCAAGATCGGCAAG
GCGGGCTACGTGACCGACCGGGGCCGCGAGAAGATCGTGAGCTGACCGAGACCACCAACAGAAGACC
GAGCTGCAGGCCATCCAGCTGGCCCTGCGAGGACAGCGGCGAGGTTGAACATCGTGACCGGAGCCAG
TACGCCCTGGGGCATCATCCAGGCCAGCCCCGACAAGAGCGAGAGCGAGCTGGTGAACAGATCATCGAG
CAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCCCCGCCCCACAAGGGCATCGGCGGCAACGAG
CAGATCGACAAGCTGGTGAAGCAAGGCATCCGCAAGGTGCTCGAATTCGAGCCCGTGGACCCCAACCTG
GAGCCCTGGAACCAACCCCGGCGAGCCAGCCCAAGACCGCCGGCAACAAGTGTACTGCAAGCACTGCAGC
TACCACTGCCTGGTGAGCTTCCAGACCAAGGGCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGAG
CGCGCAGCGCCCCCCCCAGCAGCGAGGACCAACAGAACCCCATCAGCAAGCAGCCCTGCCCCAGACC
CGCGGCGACCCACCGGCGAGCGAGGAGCAAGAAGAAGGTGGAGAGCAAGACCGAGACCGACCCCTTC
GACCCCGGGGCGGCGCAGCGGCGCAGCGAGGCGCTGCTGCAGGCCGTGCGCATCATCAAGATC
CTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACCCGCCAGGCCGACCTGAACCGCCGCCCGGC
TGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATCCTGAGCACCTGCCTGGGCGGCCCC
GCCGAGCCCGTGCCCTTCCAGCTGCCCCCGACCTGCGCTGCACATCGACTGCAGCGAGAGCAGCGGC
ACCAGCGGCACCCAGCAGAGCCAGGGCACCCAGGCGCTGGGCGAGCCCTCGAGGCCGCGCAAGTGG
AGCAAGAGCAGCATCGTGGGTGGCCCCGCGTGCAGCGCATCCGCCGACCGAGCCCGCGCGGAG
GGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCAGCGCGCCCTGACCGAGCAGCAACCGCCGCCAAC
AACGCCGACTGCGCTGGCTGGAGGCCAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCCAG
GTGCCCTTGCGCCCATGACCTACAAGGCCGCTTCGACCTGAGCTTCTTCTGAAGGAGAAGGGCGGC
CTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATCCTGGACCTGTGGGTGTACCACACCCAGGGC
TTCTTCCCCGCTGGCAGAATACACCCCGGCCCGGCGTGCCTACCCCTGACCTTCGGCTGGTGC
TTCAAGCTGGTGGCCGTGGACCCCGCGAGGTGGAGGAGGCCAACAAGGGCGAGAACAACCTGCTGTG
CACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGCGAGGTGCTGAAGTGAAGTTTCGACAGCAGC
CTGGCCCGCCGACATGGCCCGGAGCTGCACCCGAGTACTACAAGGACTGCGCCTAA

Figure 49
(Sheet 1 of 1)

rev.exon1_2.M5/10.opt_C

ATGGCCGGCCGCAGCGGCGACAGCGACGAGGCCCTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTAC
CAGAGCAACCCCTACCCCAAGCCCGAGGGCACCCGCCAGGCCGACCTGAACCGCCGCCGCGCTGGCGC
GCCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATCCTGAGCACCTGCCTGGGCCGCCCGCCGAG
CCCGTGCCCTTCCAGCTGCCCCCGACCTGCGCCTGCACATCGACTGCAGCGAGAGCAGCGGCACCAGC
GGCACCCAGCAGAGCCAGGGCACCACCGAGGGCGTGGGCAGCCCC

Figure 50
(Sheet 1 of 1)

tat.exon1_2.opt.C22/37_C

ATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCACCCCGGCAGCCAGCCCAAGACCGCCGGCAAC
AAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGCCTGGGCATCAGC
TACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCAGCAGCGAGGACCACCAGAACCCCATC
AGCAAGCAGCCCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAGGTGGAG
AGCAAGACCGAGACCGACCCCTTCGAC

Figure 51
(Sheet 1 of 1)

tat.exon1_2.opt.C37_C

ATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCACCCCGGCAGCCAGCCCAAGACCGCCTGCAAC
AAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGCCTGGGCATCAGC
TACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCCAGCAGCGAGGACCACCAGAACCCCATC
AGCAAGCAGCCCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAGGTGGAG
AGCAAGACCGAGACCGACCCCTTCGAC

Figure 52
(Sheet 1 of 1)

TatRevNef.opt.native_ZA

ATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCAACCCGGCAGCCAGCCCAAGACCGCCTGCAAC
AAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGTGCTTCCAGACCAAGGGCCTGGGCATCAGC
TACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCAGCAGCGAGGACCACCAGAACCCCATC
AGCAAGCAGCCCCCTGCCCCAGACCCGCGGGCAGCCCCACCGGCAGCGAGGAGAGCAAGAAGAAGGTGGAG
AGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGCGCCGAGCGGCGACAGCGACGAGGCCCTGCTG
CAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACCCGCCAG
GCCCCGAAGAACC CGCCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATC
CTGAGCACCTGCCTGGGCCGCCCCGCGAGCCCGTGCCTTCCAGCTGCCCCCATCGAGCGCCTGCAC
ATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCCACCGAGGGCGTGGGC
AGCCCCCTCGAGGGCGGCAAGTGGAGCAAGAGCAGCATCGTGGGTGGCCCGCCGTGCGCGAGCGCATC
CGCCGCACCGAGCCCGCCGCGAGGGCGCCGCGAGGGCGCCGCGAGGGCGTGGGCGCCGCCAGCCAG
GACCTGGACAAGCACGGCGCCCTGACCAGCAGCAACACCGCCGCAACAACGCGCGACTGCGCCTGGCTG
GAGGCCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCAGGTGCCCCCTGCGCCCCATGACC
TACAAGGCCGCTTCGACCTGAGCTTCTTCTGAAGGAGAAGGGCGGCCCTGGAGGGCCTGATCTACAGC
AAGAAGCGCCAGGAGATCCTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGACTGGCAGAAC
TACACCCCCGGCCCGCGTGCCTACCCCTGACCTTCGGCTGGTGCCTCAAGCTGGTGGCCGTGGAC
CCCCGCGAGGTGGAGGAGGCCAACAGGGCGAGAACAATGCTGCTGCACCCCATGAGCCAGCACGGC
ATGGAGGACGAGGACCGCGAGGTGCTGAAGTGAAGTTTCGACAGCAGCCTGGCCCGCCGCCACATGGCC
CGCGAGCTGCACCCCGAGTACTACAAGGACTGC

Figure 53
(Sheet 1 of 1)

TatRevNef.opt_ZA

ATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCAACCCCGGCAGCCAGCCCAAGACCGCCGGCAAC
AAGTGCTACTGCAAGCACTGCAGCTACCACTGCCCTGGTGAGCTTCCAGACCAAGGGCCTGGGCATCAGC
TACGGCCGCAAGAAGCGCCGCCAGCGCCGCAGCGCCCCCCCCCAGCAGCGAGGACCACCAGAACCCCATC
AGCAAGCAGCCCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAGGTGGAG
AGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGCGCGCAGCGGCGACAGCGACGAGGCCCTGCTG
CAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACCCGCCAG
GCCGACCTGAACCGCCGCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAGCGCATC
CTGAGCACCTGCCCTGGGCGCCCGCCGAGCCCGTGCCCTTCCAGCTGCCCCCGACCTGCGCTGCAC
ATCGACTGCAGCGAGAGCAGCGGCACCAAGCGGCACCCAGCAGAGCCAGGGCACCACCGAGGGCGTGGGC
AGCCCCCTCGAGGCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCCGCGTGCAGCGAGCGCATC
CGCCGCACCGAGCCCGCCGCGGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCACGGCGCCCTG
ACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGCCAGGAGGAGGAGGAG
GTGGGCTTCCCCGTGCGCCCCCAGGTGCCCCCTGCGCCCCATGACCTACAAGGCCGCCCTTCGACCTGAGC
TTCTTCTGAAGGAGAAGGGCGGCTGGAGGGCCTGATCTACAGCAAGAAGCGCCAGGAGATCCTGGAC
CTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCGGCCCGGCGTGCAGC
TACCCCTGACCTTCGGCTGGTGTCTCAAGCTGGTGCCTGGACCCCGCGAGGTGGAGGAGGCCAAC
AAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGCGAGGTG
CTGAAGTGGAAGTTCGACAGCAGCCTGGCCCGCCGCCACATGGCCCGCGAGCTGCACCCCGAGTACTAC
AAGGACTGCGCCTAA

Figure 54
(Sheet 1 of 1)

TatRevNefGag_C

GCCACCATGGAGCCCCTGGACCCCAACCTGGAGCCCTGGAACCAACCCGGCAGCCAGCCCAAGACCGCC
GGCAACAAGTGCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGCCTGGGC
ATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCAGCAGCGAGGACCACCAGAAC
CCCATCAGCAAGCAGCCCTGCCCCAGACCCGCGGCGACCCACCCGGCAGCGAGGAGAGCAAGAAGAAG
GTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGGCCGAGCGGGCAGACGACGAGGCC
CTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCCGAGGGCACC
CGCCAGGCCGACCTGAACCGCCGCGCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAG
CGCATCCTGAGCACCTGCCTGGGCCGCCCCGCGAGCCCGTGCCTTCCAGCTGCCCCCGACCTGCGC
CTGCACATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCACCGAGGGC
GTGGGCAGCCCCCTCGAGGCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCCGCGTGCAGCAG
CGCATCCGCGCACCGAGCCCGCGCGGAGGGCGTGGGCGCCGCCAGCCAGGACCTGGACAAGCACGGC
GCCCTGACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCCTGGCTGGAGGGCCAGGAGGAGGAG
GAGGAGGTGGGCTTCCCCTGTCGCCCCCAGGTGCCCCTGCGCCCATGACCTACAAGGCCGCTTTCGAC
CTGAGCTTCTTCTGTAAGGAGAAGGGCGGCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATC
CTGGACCTGTGGGTGTACCAACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCGGCCCGGGC
GTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACCCCGCGAGGTGGAGGAG
GCCAACAAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGC
GAGGTGCTGAAGTGGAAAGTTCGACAGCAGCCTGGCCCCCGCCACATGGCCCGGAGCTGCACCCCGAG
TACTACAAGGACTGCGAATTCGGCGCCCCGCGCCAGCATCTGCGCGGCGCAAGCTGGACGCTTGGGAG
CGCATCCGCTTGCGCCCGCGCGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAG
CTGGAGAAGTTCGCCCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAG
CTGCACCCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCCTGTAC
TGCGTGACGAGAAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAAC
AAGTGCCAGCAGAAGATCCAGCAGGCCGAGGCCGCGCCACAAGGGCAAGGTGAGCCAGAACTACCCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCAAGGCCATCAGCCCCCGCACCTGAACGCCCTGGGTGAAG
GTGATCGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACC
CCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGAC
ACCATCAACGAGGAGGCCCGCGAGTGGGACCGCGTGCACCCCGTGCACGCCGGCCCCATCGCCCCGGC
CAGATGCGCGAGCCCCGCGGCGAGCAGCATCGCCGGCACACCAGCACCTGCAGGAGCAGATCGCCTGG
ATGACCAGCAACCCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTTGGGCCCTGAACAAG
ATCGTGCGGATGTACAGCCCCGTGAGCATCTTGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGAC
TACGTGGACCGCTTCTTCAAGACCTGCGCGCCGAGCAGAGCACCCAGGAGGTGAAGAACTGGATGACC
GACACCTGCTGGTGCAGAACGCCAACCCGACTGCAAGACCATCTGCGCGCTCTCGGCCCGCGCGCC
AGCCTGGAGGAGATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGTGGCC
GAGGCGATGAGCCAGGCCAACACAGCGTGATGATGCAGAAGAGCAACTTCAAGGGCCCCCGGCGCATC
GTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCGCAACTGCGCGCCCCCGCAAGAAGGGC
TGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTGGGC
AAGATCTGGCCAGCCACAAGGGCCGCCCCGGCAACTTCTGAGAGCCGCCCGAGCCACCGCCCCC
CCCGCCGAGAGCTTCCGCTTCAGGAGACACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACC
CTGACCAGCCTGAAGAGCCTGTTGCGCAACGACCCCTGAGCCAAGCCTAA

Figure 55
(Sheet 1 of 2)

TatRevNefgagCpolIna_C

GCCACCATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCACCCCGGCAGCCAGCCCAAGACCGCC
GGCAACAAGTGTCTACTGCAAGCACTGCAGCTACCACTGCCTGGTGAGCTTCCAGACCAAGGGCCTGGGC
ATCAGCTACGGCCGCAAGAAGCGCCGACGCGCCAGCGCCCGCCAGCGAGGAGAGCAAGAAGAAG
CCCATCAGCAAGCAGCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAG
GTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGCGCCAGCGGCGACAGCGACGAGGCC
CTGCTGCAGGCGCTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACC
CGCCAGGCGGACCTGAACCGCCGCGCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAG
CGCATCCTGAGCACCTGCCTGGGCGCGCCCGCGAGCCCGTGGCCCTTCCAGCTGCCCCCGACCTGCGC
CTGCACATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCACCGAGGGC
TGGGGCAGCCCTCGAGGCGCGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGTGCGCGAG
CGCATCCGCGCGACCGAGCCCGCGCCGAGGGCGTGGGCGCGCCAGCCAGGACCTGGACAAGCAGCGG
GCCCTGACCAGCAGCAACACCGCGGCCAACAACGCGGACTGCGCCTGGCTGGAGGCGCCAGGAGGAG
GAGGAGGTGGGCTTCCCCGTGCGCCCCAGGTGCCCTGCGCCCCATGACCTACAAGGCGCCTTTCGAC
CTGAGCTTCTTCTGTAAGGAGAAGGGCGGCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATC
CTGACCTGTGGGTGTACCAACCCAGGGCTTCTTCCCCGCTGGCAGAACTACACCCCGGCGCCCGGC
GTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACCCCGCGAGGTGGAGGAG
GCCAACAAGGGCGAGAACAATGCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGC
GAGGTGCTGAAGTGAAGTTTCGACAGCAGCCTGGCCCGCGCCACATGGCCCCGCGAGCTGCACCCCGAG
TACTACAAGGACTGCCTCGAGGGCGCCCGCGCCAGCATCCTGCGCGCGCGCAAGCTGGAGCGCCTGGGAG
CGCATCCGCTGCGCCCCGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCGCGAG
CTGGAGAAGTTTCGCTTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAG
CTGCACCCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCCTGTAC
TGCGTGCACGAGAAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAAC
AAGTGCCAGCAGAAGATCCAGCAGGCGGAGGCCGCGCAAGGGCAAGGTGAGCCAGAATAACCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCGGCGGAGCAGCCCGCGACCTGAACGCTGGGTGAAG
GTGATCGAGGAGAAGGCCCTTCAGCCCCGAGGTGATCCCCATGTTACCGCCCTGAGCGAGGGCGCCACC
CCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGAC
ACCATCAACGAGGAGGCGCGGAGTGGGACCGCGTGCACCCCGTGCACGCGGCGCCCATGCCCCCGGC
CAGATGCGCGAGCCCCGCGGCGAGCAGCATCGCCGCGCACCAACAGCACCTGCAGGAGCAGATCGCCTGG
ATGACCAGCAACCCCCCATCCCGTGGGCGACATCTACAAGCGGTGGATCATCTGGGCTGAACAAG
ATCGTGCGGATGTACAGCCCCGTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGAC
TATCTGGACCGCTTCTTCAAGACCTTGCAGCGCGAGCAGACCCAGGAGGTGAAGCACTGGATGACC
GACACCTGCTGGTGCAGAACGCCAACCCGACTGCAAGACCATCCTGCGCGCTCTCGGCCCCGCGGCC
AGCCTGGAGGAGATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCCGCTGCTGGCC
GAGGCGATGAGCCAGGCCAACACAGCGTGTATGATGCAAGAGCAACTTCAAGGGCCCCCGCGCATC
GTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCGCGCCCCCGCAAGAAGGGC
TGCTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGC
AAGATCTGGCCAGCCACAAGGGCCCGCGCAACTTCTGAGAGCGCCCCGAGCCACCGCCCCC
CCCCCGAGAGCTTCCGCTTCGAGGAGACACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACC
CTGACCAGCCTGAAGAGCCTGTTGGCAACGACCCCTGAGCCAAGAATTGCGCGAGGCCATGAGCCAG
GCCACCAGCGCCAACATCCTGATGCAGCGCAGCAACTTCAAGGGCCCCAAGCGCATCATCAAGTGCTTC
AACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGAAGTGC
GGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTCCGCGAGGACCTGGCC
TTCCCCAGGGCAAGGCCCGCGAGTTCCCCAGCGAGCAGAACCGCGCCAACAGCCCCACCAGCCGCGAG
CTGACGTTGCGCGGACAACCCCGCAGCGAGGCGCGCGCGAGCGCCAGGGCACCTGAACTTCCCC
CAGATCACCTGTGGCAGCGCCCCCTGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGCCCTGCTG
GCCACCGCGCGGACGACACCGTGTGGAGGAGATGAGCCTGCCCGCAAGTGGAAGCCCAAGATGATC
GGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATCCTGATCGAGATCTGCGGCAAGAAG
GCCATCGGCGACCGTGTGATCGGCCCCACCCCGTGAACATCATCGGCGCAACATGTGACCCAGCTG
GGCTGCACCTGAACTTCCCCATCAGCCCCATCGAGACCGTGGCCGTGAAGCTGAAGCCCGGCATGGAC
GGCCCCAAGGTGAAGCAGTGGCCCCTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAG
ATGAGAAGGAGGGCAAGATCACCAAGATCGGCCCCGAGAACCCCTACAACACCCCGTGTTCGCCATC
AAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGAC
TTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCGGCTGAAGAAGAAGAAGAGCGTGACCGTGCCTG

Figure 55
(Sheet 2 of 2)

GACGTGGGCGACGCCTACTTCAGCGTGCCCC'TGGACGAGGACTTCCGCAAGTACACCGCCTTCACCATC
CCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGCTGCCCCAGGGCTGGAAGGGC
AGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCCGCAACCCGAGATC
GTGATCTACCAGGCCCCCTGTACGTGGGCAGCGACCTGGAGATCGGCCAGCACCGCGCCAAGATCGAG
GAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACCAACCCCGACAAGAAGCACCCAGAAGGAGCCCCC
TTCCCTGCCCATCGAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCGAGCTGCCCAGAGAAGGAGAGC
TGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACCCCGGCATC
AAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCCAAGGCCCTGACCGACATCGTGCCCCTGACCGAG
GAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGCGCGAGCCCGTGCACGGCGTGTACTACGAC
CCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAGTGGACCTACCAGATCTACCAG
GAGCCCTTCAAGAACCCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAG
CAGCTGACCGAGGCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCCAAGTTC
CGCTTGCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCC
GAGTGGGAGTTCTGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATC
GGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCAAGATCGGCCAAGGCCGGCTACGTG
ACCGACCGGGCCGGCAGAAGATCGTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCC
ATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGC
ATCATCCAGGCCAGCCCCGACAAGAGCGAGAGCGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAG
AAGGAGAAGGTGTACCTGAGCTGGGTGCCCGCCCAACAAGGGCATCGGCGGCAACGAGCAGATCGACAAG
CTGGTGAGCAAGGGCATCCGCAAGGTGCTGTTCTTGGACGGCATCGATGGCGGCATCGTGATCTACCAG
TACATGGACGACCTGTACGTGGGCAGCGCGGCCCTAGGATCGATTAAAAGCTTCCCGGGGCTAGCACC
GGTTCTAGA

Figure 56
(Sheet 1 of 2)

TatRevNefGagProtInaRTmut_C

GCCACCATGGAGCCCGTGGACCCCAACCTGGAGCCCTGGAACCAACCCCGGCAGCCAGCCCAAGACCGCC
GGCAACAAGTGCTACTGCAAGCACTGCAGCTACCACTGCCCTGGTGAGCTTCCAGACCAAGGGCCTGGGC
ATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCCAGCAGCGAGGACCACCAGAAC
CCCATCAGCAAGCAGCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAG
GTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCCGCGCAGCGCGACAGCGACGAGGCC
CTGCTGCAGGCCGTGCGCATCATCAAGATCCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACC
CGCCAGGCCGACCTGAACCCGCGCGCCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAG
CGCATCCTGAGCACCTGCCCTGGGCCGCCCCCGCCGAGCCCGTGGCCCTTCCAGCTGCCCCCGACCTGCGC
CTGCACATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCACCGAGGGC
GTGGGCAGCCCTCGAGGCCGCAAGTGGAGCAAGAGCAGCATCGTGGGTGGCCCGCCGTGCGCGAG
CGCATCGCCGCAACCGAGCCCGCCGCGGCTGGGGCGCCGCGCAGCCAGGACCTGGACCAAGCACCGG
GCCCTGACCAGCAGCAACACCGCCGCCAACAACGCGGACTGCGCCTGGCTGGAGGCCAGGAGGAGGAG
GAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGTGCCCTGCGCCCCATGACCTACAAGGCCGCTTCGAC
CTGAGCTTCTTCTGAAGGAGAAGGGCGGCTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATC
CTGGACCTGTGGGTGTACCACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCGGCCCGGC
GTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACCCCGCGAGGTGGAGGAG
GCCAACAAGGGCGAGAACAACCTGCCTGCTGCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGC
GAGGTGCTGAAGTGGAGTTTCGACAGCAGCTGGCCCGCCGCCACATGGCCCGCGAGCTGCACCCGAG
TACTACAAGGACTGCAAGCTTGGCGCCCGCGCCAGCATCCTGCGCGCGGCAAGCTGGACGCTTGGGAG
CGCATCCGCTGCGCCCCGCGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAG
CTGGAGAAGTTGCCCCGTAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAG
CTGCACCCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCCACCTGTAC
TGCGTGACGAGAAGATCGAGGTCCGCGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAAC
AAGTGCCAGCAGAAGATCCAGCAGGCCGAGGCCGCGGACAAGGGCAAGGTGAGCCAGAATACCCCATC
GTGCTGTAAGCTGCAGGGCCAGATGGTGACACCGCATCAGCCCCGACCCCTGAACGCTTGGTGAAAG
GTGATCGAGGAGAAGGCCCTTCAGCCCCGAGGTGATCCCCATGTTTACCGCCCTGAGCGAGGCGCCACC
CCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGAC
ACCATCAACGAGGAGGCCGCGAGTGGGACCGCGTGACCCCGTGACGCGCGGCCCATCGCCCCCGGC
CAGATGCGCGAGCCCCGCGGCGAGCAGCATCGCCGGCACCACAGCACCTTGCAGGAGCAGATCGCCTGG
ATGACCAGCAACCCCCCATCCCCGTGGGCGACATCTACAAGCGGTGGATCATCTTGGGCCCTGAACAAG
ATCGTGCGGATGTACAGCCCCGTGAGCATCCTGGACATCAAGCAGGGCCCCAAGGAGCCCTTCCGCGAC
TACGTGGACCGCTTCTTCAAGACCTTGCGCCCGGAGCAGAGCACCCAGGAGGTGAAGGCTGGATGACC
GACACCTTGCTGGTGAGAACGCCAACCCGACTGCAAGACCATCCTGCGCGCTCTCGGCCCGCGCGCC
AGCCTGGAGGAGATGATGACCGCTGCCAGGGCGTGGGCGGCCCCAGCCACAAGGCCCGCGTGCTGGCC
GAGGCGATGAGCCAGGCCAACACAGCGTGATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCATC
GTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACATCGCCCGCAACTGCCGCGCCCCCGCAAGAGGGC
TGTGGAAGTGCGGCAAGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTTGGGC
AAGATCTGGCCCCAGCCACAAGGGCCGCCCGGCAACTTCTGAGAGCGCCCCGAGCCACCGCCCC
CCCCCGGAGAGCTTCCGCTTCGAGGAGACCACCCCGGCCAGAAGCAGGAGAGCAAGGACCGCGAGACC
CTGACCAGCCTGAAGAGCCTGTTTCGGCAACGACCCCTGAGCCAGAAAGAATTCCCCAGATCACCTTG
TGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGCGGCCAGATCAAGGAGGCCCTGCTGGCCACCGGCGCC
GACGACACCGTGCTGGAGGAGATGAGCCTGCCCGCAAGTGAAGCCCAAGATGATCGGCGGCATCGGC
GGCTTCAATCAAGGTGCGCCAGTACGACAGATCCTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACC
GTGCTGATCGGCCCCACCCCGTGAACATCATCGGCCGCAACATGCTGACCCAGCTGGGTGCAACCTG
AAGTTCCTCCCATCAGCCCATCGAGACCGTGCCGTGAAGCTGAAGCCCGGCATGGACGGCCCCAAGGTG
AAGCAGTGGCCCCGTGACCGAGGAGAAGATCAAGGCCCTGACCGCCATCTGCGAGGAGATGGAGAAGAG
GGCAAGATCACCAAGATCGGCCCGGAGAACCCCTACAACACCCCGTGTTCGCCATCAAGAAGAAGGAC
AGCACCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTG
CAGCTGGGCATCCCCACCCCGCGGCTGAAGAAGAAGAAGCGTGACCGTGTGACGTGGGCGAC
GCCTACTTCAGCGTGCCCTGGACGAGGACTTCCGCAAGTACACCGCTTACCATCCCCAGCATCAAC
AACGAGACCCCGGCATCCGCTACCAAGATCCTGGAGCCCTTCCGCGCCGCAACCCCGAGATCGTGATCTACCA
TTCCAGAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCGCCGCAACCCCGAGATCGTGATCTACCA
GCCCCCTGTACGTGGGCAGCGACCTGGAGATCGGCCAGCACCGCGCAAGATCGAGGAGCTGCGCAAG
CACCTGCTGCGCTGGGGCTTACCACCCCGACAAGAAGCACCAAGGAGCCCCCTTCTGCCCCATC

Figure 56
(Sheet 2 of 2)

GAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAAC
GACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACCCCGGCATCAAGGTGCGCCAG
CTGTGCAAGCTGCTGCGCGGCGCCAAGGCCCTGACCGACATCGTGCCCCGTGACCGAGGAGGCCGAGCTG
GAGCTGGCCGAGAACC GCGAGATCCTGCGCGAGCCCGTGACGGCGTGTACTACGACCCAGCAAGGAC
CTGGTGGCCGAGATCCAGAAGCAGGGCCACGACCAAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAG
AACCTGAAGACCGGCAAGTACGCCAAGATGCGCACCGCCACACCAACGACGTGAAGCAGCTGACCGAG
GCCGTGCAGAAGATCGCCATGGAGAGCATCGTGATCTGGGGCAAGACCCCAAGTTCCGCCTGCCCATC
CAGAAGGAGACCTGGGAGACCTGGTGGACCGACTACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTC
GTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACC
TTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGATCGGCAAGGCCGGCTACGTGACCGACCGGGC
CGGCAGAAGATCGTGAGCCTGACCGAGACCACCAACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCC
CTGCAGGACAGCGGCAGCGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCC
CAGCCCGACAAGAGCGAGAGCGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTG
TACCTGAGCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAG
GGCATCCGCAAGGTGCTCTAA

Figure 57
(Sheet 1 of 1)

TatRevNef.ProtRT.opt_C

GCCACCATGGAGCCCCGTGGACCCCAACCTGGAGCCCTGGAACACCCCGGCAGCCAGCCCAAGACCGCC
GGCAACAAGTGTCTACTGCAAGCACTGCAGTACCCTGCTGGTGGAGCTTCCAGACCAAGGGCCCTGGGC
ATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGAGCGCCCCCCCCCAGCAGCGAGGACCACCAGAAC
CCCATCAGCAAGCAGCCCCCTGCCCCAGACCCGCGGCGACCCACCGGCAGCGAGGAGAGCAAGAAGAAG
GTGGAGAGCAAGACCGAGACCGACCCCTTCGACCCCGGGGCGGCGCGCAGCGGCGACAGCGACGAGGCC
CTGCTGCAGGGCCGTGCGCATCATCAAGATCTGTACCAGAGCAACCCCTACCCCAAGCCCGAGGGCACC
CGCCAGGCGGACCTGAACCGCGCCGCGCTGGCGCGCCCGCCAGCGCCAGATCCACAGCATCAGCGAG
CGCATCTCTGAGCACCTGCCTGGGCGCGCCCGCCGAGCCCGTGCCCTTCCAGCTGCCCCCCGACCTGCGC
CTGACATCGACTGCAGCGAGAGCAGCGGCACCAGCGGCACCCAGCAGAGCCAGGGCACCACCGAGGGC
GTGGGCAAGCCCCCTCGAGGCGGCAAGTGGAGCAAGAGCAGCATCGTGGGTGGCCCGCCGTGCGCGAG
CGCATCCGCGCACCGAGCCCGCGCGCGAGGGCGTGGGCGCCCGCCAGCCAGGACCTGGACAAGCAGCGC
GCCCTGACCAGCAGCAACACCGCGCCCAACAACGCGACTGCGCCTGGCTGGAGGCCCCAGGAGGAGGAG
GAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGTGCCCCCTGCGCCCCATGACCTACAAGGCGGCCCTTCGAC
CTGAGCTTCTTCTGTAAGGAGAAGGGCGGCTTGGAGGGCTGATCTACAGCAAGAAGCGCCAGGAGATC
CTGGACCTGTGGGTGTACCAACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCCGCGCCCGGC
GTGCGCTACCCCTGACCTTCGGCTGGTGTCTTAAAGCTGGTGGCCGTGGACCCCCGCGAGGTGGAGGAG
GCCAACAAGGGCGAGAACAACCTGCTGTCACCCCATGAGCCAGCACGGCATGGAGGACGAGGACCGC
GAGGTGCTGAAGTGGAGTTTCGACAGCAGCCTGGCCCGCGCCACATGGCCCGCGAGCTGCACCCCGAG
TACTACAAGGACTGCGAATTCCCCCAGATCACCTGTGGCAGCGCCCCCTGGTGAGCATCAAGGTGGGC
GGCCAGATCAAGGAGGCCCTGCTGGACACCGGCGCCGACGACACCGTGCTGGAGGAGATGAGCCTGCC
GGCAAGTGGAAAGCCCAGATGATCGGCGGCATCGGCGGCTTCATCAAGGTGCGCCAGTACGACCAGATC
CTGATCGAGATCTGCGGCAAGAAGGCCATCGGCACCGTGCTGATCGGCCCCACCCCGTGAACATCATC
GGCCGCAACATGCTGACCCAGCTGGGTGTCACCTTGAACCTTCCCCATCAGCCCCATCGAGACCGTGCCC
GTGAAGCTGAAGCCCCGCATGGACGGCCCCAAGGTGAAGCAGTGGCCCCCTGACCGAGGAGAAGATCAAG
GCCCTGACCGCCATCTGCGAGGAGATGGAGAAGGAGGCGCAAGATCACCAGATCGGCCCCGAGAACCCC
TACAACACCCCCGTGTTCGCCATCAAGAAGAAGGACAGCACCAAGTGGCGCAAGCTGGTGGACTTCCGC
GAGCTGAACAAGCGCACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCGGCTGAAG
AAGAAGAAGAGCGTGACCGTGCTGGACGTGGGCGACGCCCTACTTCAGCGTGCCCCCTGGACGAGGACTTC
CGAAGTACACCGCCCTTCAACATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAAC
GTGCTGCCCCAGGGCTGGAAGGGCAGCCCCAGCATCTTCCAGAGCAGCATGACCAAGATCTTGGAGCCC
TTCCGCGCCCCGCAACCCCGAGATCGTGATCTACCAGGCCCCCTGTACGTGGGCAGCGACCTGGAGATC
GGCCAGCACCGCGCCAAGATCGAGGAGCTGCGCAAGCACCTGCTGCGCTGGGGCTTCACCACCCCGAC
AAGAAGCACCAGAAGGAGCCCCCTTCTGCCCCATCGAGCTGCACCCCGACAAGTGGACCGTGACGCCC
ATCGAGCTGCCCCGAGAAGGAGAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGG
GCCAGCCAGATCTACCCCGCATCAAGGTGCGCCAGCTGTGCAAGCTGCTGCGCGGCGCCAAGGCCCTG
ACCGACATCGTGCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCAGCGAGATCTTGCAGGAG
CCCGTGACGGCGTGTAACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGGCCACGAC
CAGTGGACCTACAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAAGTACGCCAAGATGCGC
ACCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCAAGAAGATCGCCATGGAGAGCATCGTG
ATCTGGGGCAAGACCCCCAAGTTCCGCTGCCCCATCCAGAAGGAGACCTGGGAGACCTGGTGGACCGAC
TACTGGCAGGCCACCTGGATCCCCGAGTGGGAGTTCTGTAACACCCCCCCCCCTGGTGAAGCTGTGGTAC
CAGCTGGAGAAGGAGCCCATCATCGGCGCCGAGACCTTCTACGTGGACGGCGCCGCAACCGCGAGACC
AAGATCGGCAAGGCCGGCTACGTGACCGACCGGGGCGCGCAGAAGATCGTGAGCTGACCGAGACCACC
AACCAGAAGACCGAGCTGCAGGCCATCCAGCTGGCCCTGCAGGACAGCGGCAGCGAGGTGAACATCGTG
ACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCGACAAGAGCGAGAGCGAGCTGGTGAAC
CAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCTGAGCTGGGTGCGCCGCAAGGGCATC
GGCGGCAACGAGCAGATCGACAAGCTGGTGAGCAAGGGCATCCGCAAGGTGCTCTAA

FIGURE 58 (SEQ ID NO:61)

atgagagtgatggggacacagaagaattgtcaacaatgggtggatatggggcatcttaggc
ttctggatgctaattgatttgaacacggaggacttgtgggtcacagtctactatggggta
cctgtgtggagagacgcaaaaactactctatttctgtgcatcagatgctaaagcatatgag
acagaagtgcataatgtctgggtacacatgcctgtgtacccacagaccccaaccacaa
gaaatagtttgggaaatgtaacagaaaattttaatatgtggaaaaatgacatggcagat
cagatgcatgaggatgtaatcagtttatgggatcaaagcctaagccatgtgtaaagttg
acccactctgtgtcactttaaactgtacagatacaaatgttacaggtaatagaactgtt
acaggtaatagtagcaataatacaaatgggtacaggatattataacattgaagaaatgaaa
aattgctctttcaatgcaaccacagaattaagagataagaaacataaagagtatgcactc
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ataaattgcaataacctcaaccataacacaagcctgtccaaagggtctcttttgaccgatt
cctatacattactgtgtccagctgggttatgagattctaaagtgtataataagacattc
aatgggacaggaccatgttataatgtcagcacagtacaatgtacacatggaattaagcca
gtgggtatcaactcaattactgtttaatggtagtctagcagaagaaggataataattaga
tctgaaaatttgacagagaataccaaaacaataatagtacaccttaatgaatctgtagag
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gcattctatgcaacaaatgatgtaataggaaacataagacaagcacattgtaacattagt
acagatagatggaacaaaactttacaacaggtaatgaaaaaattaggagagcatttccct
aataaaacaataacaatttaaacacatgcaggaggggactctagaaattacaatgcatagc
tttaattgtagaggagaatttttctattgtatacatcaaactgtttaatagcacatac
cactctaataatgggtacatacaaatacaatggtaattcaagctcacccatcacactccaa
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cccattgcaggaaacataacatgtatagatcaaacatcacaggaaatactattgacacgtgat
ggaggatttaacaccacaaacaacacagagacattcagacctggaggaggagatatgagg
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gcacccactaaggcaaaaagaagagtggtgcagagagaaaaaagagcagtggaatagga
gctgtgttccttgggttcttgggagcagcaggaagcactatgggcgcagcgtcaataacg
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caggcgagagtcctggctatagaaagatacctaaaggatcaacagctcctagggtattgg
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aattacacaggcttaatatacaatttgcttgaagactcgcaaaaccagcaggaaaagaat
gaaaaagattttattagaattggacaagtggacaactctgtggaattgggttgacatatca
aactggccgtgggtatataaaaatattcataatgatagtaggaggcttgataggtttaaga
ataatttttgctgtgctttctatagtgaatagagtttaggcagggaatactcacctttgtca
tttcagacccttaccccaagcccgaggggactcgcagagctcgagggaatcgaagaagaa
gggtggagagcaagacagagacagatccatacgattgggtgagcggattcttgtcgcttgcc
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agtgtctattagctgtcttgataccatagcaataacagtagctgaaggaacagataggatt
atagaattagtacaaagaatttgtagagctatcctcaacatacctagaagaataagacag
ggctttgaagcagctttgctataa

FIGURE 59 (SEQ ID NO:62)

atgagagtgtatggggacacagaagaattgtcaacaatgggtggatatggggcatcttaggc
ttctggatgctaataatgatttgaacacggaggacttgtgggtcacagtctactatgggta
cctgtgtggagagaagcaaaaactactctattctgtgcatcagatgctaagcatatgag
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aatgggacaggaccatgttataatgtcagcacagtacaatgtacacatggaattaagcca
gtggtatcaactcaactactgttaaatggtagtctagcagaagaaggataataattaga
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gcattctatgcaacaaatgacgtaataaggaacataagacaagcacattgttaacattagt
acagatagatggaataaaaactttacaacaggtaatgaaaaaattaggagagcatttccct
aataaaacaataaaaatttgaaccacatgcaggaggggatctagaaattacaatgcatagc
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taccctaagaatggtacatacaaatacaatgtaattcaagcttaccatcacactccaa
tgcaaaataaaacaaattgtacgcatgtggcaagggtaggacaagcaatgtatgccct
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gggggatttaacaacacaaacaacgacacagagagacattcagacctggaggaggat
atgaggataaactggagaagtgaattatataaataaaagtggtagaaattaagccattg
ggaatagcaccactaaggcaaaaagaagagtgtgcagagaaaaaaagagcagtggga
ataggagctgtgttccctgggttcttgggagcagcaggaagcactatgggagcagcgtca
ataacgctgacgggtacaggccagacaactgttgtctggtatagtgcacagcaaagcaat
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cagctccaggcgagagtcctggctatagaaagatacctaaggatcaacagctcctaggg
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attaataattacacagaaacaatattcaggttgcttgaagactcgcaaaaccagcaggaa
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gaagaagggtggagagcaagacagagacagatccatacgattgggtgagcggattcttgtcg
cttgcctgggacgatctgaggagcctgtgcctcttcagctaccaccgcttgagagacttc
atattaattgcagtgagggcagtggaacttctgggacacagcagcttcaggggactacag
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aaaaagagtgtatttagtccgcttgataccatagcaatagcagtagctgaaggaacagat
aggattatagaattggtacaaagaattttagagctatcctcaacatacctaggagaata
agacagggtcttgaagcagcttctgctataa

FIGURE 60 (SEQ ID NO:63)

atgagagcgagggggatactgaagaattatcgacactgggtggatatggggcatcttaggc
 ttttggtgctaataatgatgtgtaattgtgaaggcttgtgggtcacagtctactacggggta
 cctgtggggagagaagcaaaaactactctattttgtgcatcagatgctaagcatatgag
 aaagaagtgcataatgtctgggtacacatgcctgtgtacccacagaccccaaccacaa
 gaagtgattttgggcaatgtaacagaaaattttaacatgtggaaaaatgacatgggtggat
 cagatgcaggaagatataatcagtttatgggatcaaagccttaagccatgtgtaaaattg
 accccactctgtgtcacttttaactgtacaaatgcaactgttaactacaataatacctct
 aaagacatgaaaaattgctctttctatgtaaccacagaattaagagataagaaaaagaaa
 gaaaatgcacttttttatagacttgatatagtaccacttaataataggaagaatgggaat
 attacaactatagatttaataaattgtaatacctcagccataacacaagcctgtccaaaa
 gtctcgtttgacccaattcctatacattattgtgtccagctgggttatgcgctctaaaa
 tgtaataataagaaattcaatggaaataggaccatgagataatgtcagcacagtacaatgt
 acacatggaaattaagccagtgggtatcaactcaattactgttaaatggtagcctagcagaa
 gaagagataataattagatctgaaaaatctgacaaacaatgtcaaaacaataatagtacat
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 agaattaggaccaggacaagcattctatgcaacaggagacataataggagataaagaca
 gcacattgtaacattagtaaaaaatgaatggaatacaactttacaagggttaagtcaaaaa
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 ctagaaattactacacatagctttaattgtggaggagaatttttctattgcaatacaaca
 gacctgtttaatagtagacatagtaatggtacatgcactaatggtacatgcatgtcta
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 cagtgggatagggaaattagtaattacacaaacacaatatagaggttgcttgaagactcg
 caaagccagcaggaagaaatgaaaaagatttactagcattggacaggtggaacaatctg
 tggaaattggttttagcataacaaattggctgtggtatataaaaaatttcataatgatagta
 ggaggcttgataggtttaagaataatttttctgtgtctctctagtaaatagagttagg
 cagggatactcacccttgctcattgcagacccttatcccaaacccgaggggacccgacagg
 ctcgagggaatcgaagaagaagggtggagagcaagacagcagcagatccattcgattagt
 agcggattcttgacacttgctgggacgacctacgaagcctgtgctctctctgtaccac
 cgattgagagacttcataattaattgtagttagagcagtggaacttctgggacacagtagt
 ctgagggactgcagaggggggtggggaacccttaagtatttggggagtcttgtgcaatat
 tggggtctagagttaaaaaagagtgtctattaatctgcttgatactatagcaatagcagta
 gctgaaggacagataggattctagaattcatacaaaaacctttagtagggtatccgcaac
 gtacctagaagaataagacagggttcgaagcagcttgcataa

FIGURE 61 (SEQ ID NO:64)

atgagagtggaggggatactgaggaattggcaacaatggtggatatggggcatcttaggc
ttttggatgttaattgatttagtgatttggggaacttgtgggtcacagtctattatggg
gtacctgtgtggaagaagcaaaaactactctattctgtgcatcagatgctaaagcatat
gagagagaagtgcataatgtctgggctacacatgcctgtgtgcccacagacccaaccog
caagaaatggtcttgggaaatgtaacagaaaattttaacatgtggaaaaatgataggtg
gatcagatgcatgaggatataatcagtttatgggatcaaagcctaaagccatgtgtaaag
ttgacccactctgtgtcacttttagagtgtataacgttaatactaccaatgaaatgaca
aattgctctttcaatgcaaccacagacgtaagagataagaaacagagagtgtctgcattt
ttttatagacttgatagtagtaccacttaatgagaataacaatgaatcccagaagtataga
ttaataagttgcaatacctcaaccataacacaagcctgtccaaaggctcacttttgacca
attcctatacattactgtactccagctggttatgcgatttctaaagtgtataataagaca
ttcaatgggacaggaccatgccataatgtcagcacagtacaatgtacacatggaattag
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agatctgaaaaatctgacaaaacatgccaaaataataatagtacaccttaatgaatctgta
gaaattgtgtgtacaagacccaacaataatacaagaaaaagtataaggataggacggga
caaacattctatgcaacaaatggcataataggaaacataagacaagcacattgtaacatt
agtgaagagagatggaacaaaaccttacaacaggtaggaaaaaattagcagaacacttc
cctaataaaaacaataaagtttgaaccatcctcaggaggggatctagaaattactacacat
agctttaattgtggaggagaatttttctattgcaatacatcaggcctgtttaatggtaca
tacaatcacactacagaaggttaattcaactcaaccatcacactcccattgcagaataaaa
caaattataaacatgtggcgaggtaggacgagcaatgtatgctcctccattgcagga
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cggagaagtgaattatataaataaaagtggtagaaattaagccattgggaatagcacc
actggggcaaaaaggagagtggtggagagagaaaaagagcagtgggactaggagctatg
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agagtcctggctatagaaagatacctaaaggatcaacagctcctagggctttggggctgc
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acaaacacaatatagaggttgcttgaagactcgcaaacccagcaggaacaaaatgaaaa
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gcccttaagtatctaggaagtcttgtgcagtattggggcttggaactaaaaaagagtgtct
gttagtctgcttgataccgtagcaatagtagtagctgaaggaacagataggattatagaa
ttagtacaagagtttgtagagctatccgcaacatacctacaagaatcagacagggttt
gaaacagctttgctataa

FIGURE 62 (SEQ ID NO:65)

atgagagtgagggagataccgaggaattggcaacaatgggtggatatggggaatcttaggc
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gtacctgtgtggaagaagcaaaaactactctatttctgtgcatcagatgctaaagcatat
gagaacgaagtgcataatgtctgggtacacatgcctgtgtacccacagaccccaaccca
caagaaatagttttggaatgtaacagaaaattttaacatgtggaatgacatgggtg
gatcagatgcatgaggatataatcagtttatgggatcaaagcctacagccatgtgtaaa
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gactctagcaacttttagtgagtataagattaataaattgtaatacctcagccatgacaca
gcctgtccaaaggctcacttttgaccaattcctatacattattgtgctccagctggttat
gcatcttaagtgtataataaagacatttaattgggacaggaccatgcagtaattgtcagc
acagtacaatgtacacatggaattaagccagtggtatcaactcaactcctgttaaatggt
agcctagcagaaaaagaaataataattagatccgaaaatctgacaaacaatgtcaaaaca
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agaaaaagtataaggataggaccagacaaacattctatgcaacaggagaaataatagga
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FIGURE 63 (SEQ ID NO:66)

gtcgacaagagcagaagacagtggaatgagagtgcggggatactgaggaattacccac
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cagatgctaaagcatatgataaagaagtgcataatgtctgggccacacatgcctgtgtac
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FIGURE 64 (SEQ ID NO:67)

gtcgacaagagcagaagacagtggaatgagagtgaggggatactgaggaattatccac
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taccacagatcccaaccacagaattagtttggaaaatgtaacagaaaattttaaca
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cggaacttctgggacacagcagtcctcaggggactacagaggggtgggaaatccttaagt
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gaatttggagagctatccgcaacatacctacaaggataagacagggtttgaagcagctt
tgcaataactctagaaagaaacaaggcggaattc

FIGURE 65 (SEQ ID NO:68)

atgagagtgcggggatactgaggaattatccacaatggtggatatgggtcatcttaggc
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gaagtgcataatgtctgggccacacatgctgtgtacccacagatcccaaccacaagat
ttggttttggaaaatgtaacagaaaattttaatatgtggaaaatgacatggtggatcag
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ccactctgtgtcactttaaattgtaaagcaaatgttactgttaaaactaatgcaaatgtt
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gcacaatattggggcttagaactcaaaaagagtgtatttagtctgcttgacatcacagca
attgcagtagctgaaggaacagatagaattatagaattaatacaagaatttggagagct
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FIGURE 66 (SEQ ID NO:69)

atgagagtgagggggatactgaggaattatcaacaatgggtggatatggggccagcttaggc
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gtacctgtgtggaaagaagcaaaaactactctattctgtgcatcagatgctaaaggatat
gaaaaagaagtgcataatgtctgggtcacacatgcctgtgtacccacagaccccaaccca
caagaactgggtgtggaaaatgtaacagaaaattttaacatgtggaaaaatgacatggta
gatcagatgcatgaggatataatcagtttatgggaccaaaagcctaaagccatgtgtaaag
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ttaaatggtagcctagcagaaaaagagataataattaaatctaaaaatctgacaaaacat
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ataataggagatataagacaagcacactgtaacattagcgaaagtaaatggaataaaact
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tgcgacgtcggggcca

FIGURE 67 (SEQ ID NO:70)

atgagagtgaggggatactgaggaattatcaacaatggtggatatgggccagcttaggc
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caagaactggttggtggaaaatgtaacagaaaattttaacatgtggaaaatgacatggta
gatcagatgcatgaggatataatcagtttatgggaccaaagcctaaagccatgtgtaaag
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ggccttgtag

FIGURE 68 (SEQ ID NO:71)

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aggaaatgagaaattgtacttttaataataaccacagaaataacagataagaaaaagcaag
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gggcgaattc

FIGURE 69 (SEQ ID NO:72)

gtcgacaagagcagacgacagtggcaatgagagtgatgggaatactgaggaattgtccac
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tctgtgcactctgatgctaaagcatatgagagggaggtgcataatgtttgggctacacatg
cctgtgtaccacagaccccaacccacaagaaatagtattggaaaatgtaacagaaaatt
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c

FIGURE 70 (SEQ ID NO:73)

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FIGURE 71 (SEQ ID NO:74)

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ggcggaattcc

FIGURE 72 (SEQ ID NO:75)

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ggcggaattcc

FIGURE 73 (SEQ ID NO:76)

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gatcagatgcatgaggatataatcagtttatgggatcaaagtctaaaacatgtgtaaag
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taa

FIGURE 74 (SEQ ID NO:77)

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gacagttggaaagatctgtggacttggtttgacatatcaaagtggttgtggtatataaga
atattcatcatgatagtaggaggttgataggtttaagaataatttttaggtgtgctctct
atagtgaagaggttaggcagggatactcaccttgcgtttcagacccttatcccaaac
ccgaggggaacccgacaggctcagaggaatcgaagaagaaggtggagagcaagacaaagac
agatcaattcgatttagtgagcggattcttagcacttgcctgggacgacctgcggagcctg
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ggaaatcttgtgcagtattggggtctggaactaaaaaagagtgtattagtctgcttgat
accatagcaatagcagtagctgaaggaaacagataggattgttgaaataatacagagaatt
gttagagctatccgcaacatacctagaagaataagacagggttgaagcagctttagcta
taa

FIGURE 75 (SEQ ID NO:78)

gtcgacaagagcagaagacagtggcaaggagtgagggggatagagaggaattggcaacaa
tgggtggatatggggcatcttaggcttttggatgttaatgatttgtaatgtgttgggaaac
ttgtgggtcacagtgtattatggggtacctgtgtggaaagaagcaataactactctattc
tgtgcatcaaagtctaaagcatatgagagggaggtgcataatgtctgggctacacatgcc
tgtgtaccacagaccccaaccacagaagaaatagtttgggaaatgtaacagaaaatttt
aatatgtggaaaaatgacatgggtggatcaaagtcatgaggatataatcagtttatgggat
caaagcctaaagccatgtgtaaagttgacccactctgtgtcacttttagaatgtacaggg
gttaaggctaccaataatagtagtgccaccaatagtagtaatgttaccaacaatgatgaa
ataaaaaattgctcttttcaatgcaaccacagaaataaaagacaagaagcacaagagtat
gcacttttttataggctcgatatagtaccacttaataatggcaaccctagttagggcaat
tctagtggagaagtatagattaataaattgttaatacctcaaccttaacacaagcctgtcca
aaggtctcttttgacccaattcctatacattattgcactccagctgggttatgagattcta
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tgtacacatggaaataaaccagtggtatcaactcaactactgttaaatggtagcttagca
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cagcttaataaaattgttagaaattgtgtgcacaagacccggcaataatacaagaaaagt
gtaaggtagaggaccaggacaacattctatgcaacaggtgacataataggagacataaga
caagcacattgtaacattactgaagataagtggaatgaaactttacaatgggtaggtaaa
aaattaggagagctcttccctaataaaaacaatagaatttaagccatcctcaggaggggac
ctagaaattacaacacatagctttaaattgttagaggagaatttttctattgcaatacatca
caactatttaatagtagacatacaattctacacaaatgcataatgatacaggaagttaattca
accatcacactcccattgcaaaaataaagcaaatataaacatgtggcagggggtaggacgg
gcaatgtatgcccctcccattgcaggaaacataacatgtaaatcaaatattacaggaata
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attaagccattgggaatagcaccactgaagcaaaaaggagagtggtaggagagagaaaaa
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ggcgacgctcaataacgctgacggtacaggccaggcaattgttgtctggcatagtgcac
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cagctcctaggactttggggctgctctggaaaactcatctgcaccactactgtgccttgg
aactcaagttggagtaataaatctctaactgatatttgggataacatgacatggatgcag
tgggtagagaaattaataattacacaaccacaataataaccagttgcttgaaaaatcgcaa
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ggcttgataggtttaagaataatttttgcctgtgctatctatagtaaacagagtttaggcag
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ggattcttctcacttgcttgggacgatctgcggaacctgtgcctcttcagctaccaccga
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gaaacccttaaatatctaggaagtcttgggcagtagttggggcttgggaactaaaaaagagt
gctatttagctgcttgatgccatagcaatagcagtagctgagggaaacagataggattata
gaattcatacaaagaattttaggggctatccgcaacacacctagaagaataagacatggc
tttaagcagctttgcaataactctagaaagaacaagggcgaattcc

FIGURE 76 (SEQ ID NO:79)

gtcgacaagagcagaagacagtggcaatgagagtgagggggatacagaggaattggcaac
aatgggtggatatggggcatcctaggccttttggatgttaatgatttgtaatgtgttggaa
acttgtgggtcacagtgtattatgggggtacctgtgtggaaagaagcaaaaactactctat
tctgtgcatcagatgctaaagcatatgagagggaggtgcataatgtctgggctacgcatg
cctgtgtacccacagaccccaaccacaagaaatagttttgggaaatgtaacagaaaatt
ttaatatgtggaaaaatgacatgggtggatcaaatgcatgaggatataatcagtttatggg
atcaaagcctaaagccatgtgtaaagttgaccccaactctgtgtcacttttagaatgtacag
gggttaaggctaccaataatagtagtgccaccaatagtagtaatgttaccaacaaagatg
aaataaaaaattgctctttcaatgcaaccacagaaataaaagacaagaagcacaagagt
atgcacttttttataggtcgatatagtaccacttaataatggcaaccctagtggggca
attctagtggagaagtagattaacaaattgtaatacctcaaccttaacacaagcctgtc
caaagggtctcttttgacccaattcctatacattattgcactccagctgggttatgcatc
taaagtgtataataaagacattcaatgggacaggaccatgccataatgtcagtagcagtag
aatgtcacatggaattaaaccagtgggtatcaactcaactactgttaaatggtagcttag
cagaagaagagataataattagatctgaaaatctgacaaacaatgctaaaataataatag
tacagcttaataaatctgtagaattgtgtgcacaagaccggcaataatacaagaaaa
gtgtaaggataggaccaggacaacattctatgcaacaggtgacataataggagacataa
gacaagcagcatgtgaacattactgaagataaatggaatgaaactttacaatgggtaggta
aaaaattaggagagctcttccctaataaaacaatagaatttaagccatcctcaggagggg
acctagaataatacacacatagctttaattgttagaggagaggtttttctattgcaatacat
cacaactatttaatagtacatacaattctacacaaatgcataatgtacaggaagtaatt
caaccatcacactcccctgcaaaaataaagcaaattataaacatgtggcaggggtaggac
gggcaatgtatgcccctcccattgacaggaacataaactgtaaatcaaatattacaggaa
tactatttagtagctgtatggaggcaacacaaatgacacaaatggcacagaaatattcagac
ctggaggagagatatgaaggacaattgggagaagtgaattatataaatataaagtggtag
aaattaagccattgggaatagcaccactgaagcaaaaaggagagtggtggagagagaaa
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tggggcgagcgtcaataacgctgacgggtacaagccaggcaattgttgtctggcatagtgc
aacagcaagcaatttgcctgagagctatagaggcgcaacagtatatgttgcaactcacgg
tctggggcattaaagcagctccaggcaagagtcctggctatagaaagatacctacaggatc
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ggaactcaagttggagtaataaatctcttaactgatatttgggataacatgacatggatgc
agtgggtagagaaaattaataattacacaaccacaatataccagttgcttgaaaaatcgc
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ggaattgggttagcataacacagtggtatgtatataaaaaatattcatcatgatatagtag
gaggttgataggtttaagaataatttttgcctgtgctatctatagtaaacagagttaggc
agggatactcacctctgtcatttcagacccttaccocaaacccgaggggacccgacaggc
tcggaagaatcgaagaagaaggtggagagcaagacagagagagatccattcgattagtga
gcggtattcttctcacttgccttgggacgatctgcggaacctgtgcctcttcagctaccacc
gattgagagacttcatattgattgtgacgagagtggtggaacttctggggcgaggggggt
gggaaaccccttaaatatctaggaagtcttgggcagtattggggcttggaactaaaaagga
gtgctatttagtctgcttgatgccatagcaatagcagtagttgaggggaacagataggatta
tagaattcatacaaagaattttagggctatccgtaaacacacctagaagaataagacagg
gctttgaaagcagctttgcaataactctagaagaacaaagggcgaaattcc

FIGURE 77 (SEQ ID NO:80)

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atgagagtga tggggatcaa gaggaattgt caacaatggt ggatatgggg catcttaggc
ttttgggtgc ttatgatttg taatgtaatg ggggaacttg gggtcacagt ctattatggg
gtacctgtgt ggagagaagc aaaaactaca ctattctggg catcagatgc taaagcatat
gagaaagaag tgcataatgt ttgggctaca catgcctgtg taccacaga cccaaccca
caagaaatag ttttggaata tgtaacagaa aattttaaca tgtgggaaaa taacatggta
gaccagatgc atgaggatat aatcagttta tgggatcaaa gtctaaaacc atgtgtaaag
ttgaccccac tctgtgtcac tttaaattgt agaaatgtaa cggttactac taacaatgat
aataatgtta cttacaataa tagcatacct gaagaaataa aaaattgctc tttcaatata
accacagaaa taagagacaa gaaaaagata gaatatgcac ttttttatag acttgggtata
gtaccgctta aggagaacaa acttaattcc agtgagtata gattaataaa ttgtaatacc
tcagccataa cacaagcctg tccaaaggct tcttttgacc caattcctat acattattgt
gctccagctg gttatgcgat actaaagtgt aataataaga cattcaatgg aacaggacca
tgcaataatg tcagcactgt acagtgtaca catggaatta agccagtggg atcaactcaa
ctactgttaa atggtagtct agcagaggaa gagataataa ttagatctaa aaatatgaca
aacaatgtca aaacaataat agtacatctg aatgaatctg tagaaattgt gtgtacaagg
cccaataatca atacaagaag aagtatgagg ataagaccag gacaaacatt ctatgcaaca
ggagaaataa taggagacat aagacaagca tattgtaaaa ttagtgaaga tcaatggaat
aaaactttac gcagggttaag tgaaaaatta agagaacact tccctgataa aacaataaaa
tttgaaccac cctcaggagg agacttagaa attacaacac atagctttaa ttgtagagga
gaatTTTTCT attgcaatac atcagaactg tttaatagta catacatgcc taatggtaca
gaaagtaata caagcaaaac catcactctc ccatgcagaa taaaacaaat tataaatatg
tggcaggggg taggacgagc aatgtatgcc cctccattg caggaaacat aacatgtcaa
tcaaataatca caggaatact attgaccctg gatggaggag aagagtcaaa gtcaaattgga
acagagatat tcaggcctgc aggaggggat atgaaggaca attggagaag tgaattatat
agatataaag tggtagaaat taaaccatta ggagtagcac ccactgaggc aaaaaggaga
gtggtggaga gagaaaaaag agcagtggga ataggagctg tgttccttgg gttcttggga
gcagcaggaa gcactatggg cgcggcgtca ataacgctga cggtagaggc cagacaaccg
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atgttgcaac tcacagtctg gggcattaag cagctccaga caagagtcct ggctgtagaa
agatacctaa aggatcaaca gctcctaggg ctttggggct gctctggaaa actcatctgc
accactgccg tgcttggaa ctccagtgg agtaataagt ctcaaacaga tatttgggat
aacatgacat ggatgcagtg ggatagagag atcagtaact acacagaaac aatatacaag
ttgcttgaag actcgcaaaa ccagcaggaa caaatgaaa aggatttact agcattggac
agttggaaaa atctgtggaa ttggtttgat ataacaaaat ggctgtggta tataaaaata
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ataaatagag ttaggcaggg atactcacct ttgtcattac agacccttac cccaaacccg
aggggaccag acaggctcgg aagaatcgaa gaagaagggt gagagcaaga cagagacaga
tccgtgagat tagtgaacgg attcttagca cttgtctggg acgacctgcg gagcctgtgc
ctcttcagct accaccaatt gagagactta atattgattg tagcgagagc agtggaagtt
ctgggacgca acagtctcag gggactacag acggggtggg aagctcttaa gtatctggga
aaccttgtgc tgtattgggg tctggagctg aaaaggagcg ctattagtct gttggatata
acagcaatag tagtagctga aggaacagat aggatTTTTG aagcaatatg cagaatttgt
agagctatcc gtaacatacc tagaagaata agacggggct ttgaagcagc tttgtctata

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FIGURE 78 (SEQ ID NO:81)

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ggatccacta gtaacggccg ccagtgtgct ggaattcgcc cttccacgcg tcgacaagag
cagaagacag tggcaatgag agtgcagggg atactgagga attgtcaaca atggtggaca
tggggcatct taggcttttg gataataatg acttgtaatg tgggtgggaa cttgtgggtc
acagtttatt atgggggtacc tgtgtggaaa gaagcaaaaa ctactctatt ctgtgcatca
gatgctaaag catatgagaa agaagtgcac aatgtttggg ctacacatgc ctgtgtaccc
acagacccca acccacaaga aatagttttg gaaaatgtaa cagaaaattt taatatgtgg
aaaaatgata tgggtgatca gatgcatgag gatgtaatca gtttatggga ccaaagccta
aagccatgtg taaagttgac cccactttgt gtcactttaa attgtacaga tgttgataaa
aatagtactg aaatgtatag gaaaaccaca aatgataatg gtaatgatac catagataga
gaaatgaaaa attgctcttt caatgcaacc acagacatac aagataagaa aacgggagtg
tatgcacttt tttatcgact ggatatagta ccactcaatg atactaaca ctctaggag
tatagattaa taaattgtaa tacctcaacc atgacacaag cctgtccaaa ggtctctttt
gatccaattc ctatacatta ttgtactcca gctggttatg cgattctaaa gtgtaataat
aagacattca gtgggacggg accatgcaat aatgtcagca cagtacaatg tacacatgga
attaagccag tgggtatcaac tcaactactg ttaaattgga gcctagcaga aaaagagata
ataattagat ctaaaaatct gacagacaat gccaaaacaa taatagtaca tcttaatgaa
tctatagcaa ttatgtgtac aagacctggc aataatacaa gaaaaagtat aaggatagga
ccaggacaag cattctttgc aacaggagca ataataggag atataagaaa agcatattgt
aacattagcg aaggtgaatg gaatagaact ttacaaaggg taggtagaaa attagcagaa
cacttccctg gtaaaagaat aagatttgca ccaccttcag gaggggacct ggaaattaca
acacatagct ttaattgtgg aggagaattt ttctattgca atacaacaca actgtttaat
aggacataca atacaacaca actgtttaat ggtacataca gctctaacga tacagaaagt
aatttcacac tcccatgcag aataaaacaa attataaaca tgtggcagga ggtaggacga
gcaatgtatg ctctctctat aaaaggaaac ataacatgta actcaaatat cacaggatta
ctgttggtgc gtgatggagg agaagacaat aacacagaaa atgacacaga gaccttcaga
cctggaggag gagatatgag ggacaattgg agaagtgaat tatacaataa taaagtggta
gaaattaagc cattgggaat agcacctact ggggcaaaaa ggagagtggg ggagagagaa
aaaagagcag tgggaatagg agctgtgttc cttgggttct tgggagcagc aggaagcact
atgggcgcgg cgtcaataac gctgacggtg caggccagac aattattgtc tggtatagt
caacagcaaa gcaatttgct gagggccata gaggcgcaac aacatatgtt gcaactcaca
gtctggggca ttaaacagct ccagacaaga gtattggcca tcgaaagata cctaaaggat
caacagctcc taggaatttg gggctgctct ggaaaactca tctgcaccac tgctgtgcct
tggaactcca gttggagtaa tagaactgag ggagatatat ggaataacct gacctggatg
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caaaaccagc aggaacaaaa tgaaaaggat ttattggcct tgagcaattg gcaaaatctg
tggagttggg ttaacatatc aaattggctg tggatatata gaatttcat aatgatagta
ggaggcttga taggtttaag aataattttt gctgtgctct ctttagtgaa taaagttagg
cagggatact cacctttgtc gttgcagacc cttaccccg aaccaagggg acccgacagg
ctcagaggaa tcgaagaaga aggtggagag caagacagag acagatccgt tcgattagt
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agtgtctatta atctgcttga tactatagca atagcagtag ctgaaggaac agataggatt
atagaattaa tactaggact tggtagagct atctgcaaca tacctagaag aataagacag
ggctttgaag cagctttgca ataactctag actagctaag ggcgaattct gcagatatcc
atcacactgg cggccgcg

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FIGURE 79 (SEQ ID NO:82)

atgggtgcga gagcgtcaat attaagcggc ggaaaattag ataaatggga aagaattagg
ttaaggccag ggggaaagaa acatttatatg ttaaaacatc tagtatgggc aagcaggagag
ctggaaagat ttgcacttaa ccctggcctg ttagaaacat cagaaggctg taaacaaata
ataaaacagc tacaaccagc tcttcagaca ggaacagagg aacttagatc attattcaac
acagtagcaa ctctctattg tgtacataaa gggataaagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa tgtcagcaaa aagcacagca ggcaaaagcg
gctgacgaaa aggtcagtca aaattatcct atagtacaga atgcccagg gcaaatggta
caccaagcta tatcacctag aacattgaat gcatgggtaa aagtaataga ggagaaggct
ttcaaccagc aggtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgttaaatac agtgggggga catcaagcag ccatgcaa atgttaaagat
accatcaatg aggaggctgc agaattgggat aggacacatc cagtgcagtc agggcctgtt
gcaccaggcc agatgagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa tagcatggat gacaagtaat ccacctatc cagtaggaga catctataaa
agatggataa ttctgggggt aaataaaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aagggccaaa agaacccttt agagattatg tagatcggtt ctttaaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggcttcatta
gaagaaatga tgacagcatg tcaggagtg ggaggacct gccataaagc aagggtgttg
gctgaggcaa tgagccaaac aaacagtaac atactagtgc agagaagcaa ttttaaggc
cctaacagaa ttgttaaatg tttcaactgt ggcaaagtag ggcacatagc cagaaagtgc
agggccccta ggaaaaagg ctgttggaat tgtggacagg aagggcacca aatgaaagac
tgtactgaga ggcaggctaa ttttttaggg aaaatctggc cttcccacaa ggggaggcca
gggaatttcc tccagaacag accagagcca acagcccac cagcagagcc aacagccca
ccagcagaga gcttcagggt cgaggagaca accccgtgc cgaggaaagga gaaagacagg
gaacctttaa cttccctcaa atcactcttt ggcagcgacc cctcgtcaca ataa

FIGURE 80 (SEQ ID NO:83)

atgggtgcga gagcgtcaat attaagcggc ggaaaattag ataaatggga aagaattagg
ttaaggccag ggggaaagaa acattatatg ttaaaacatt tagtatgggc aagcagagag
ctggaaagat ttgcacttaa ccctggcctg ttagagacag cagaaggctg taaacaaata
ataaaacagc tacaaccagc tcttcagaca ggaacagagg aacttagatc attattcaac
acagtagcaa ctctctattg tgtacataaa ggaatagagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa tgtcaacaaa aggcacaaca ggcaaaagcg
gctgatgaaa aggtcagtca aaattatcct atagtacaga atgccaaggg gcaaattggta
caccaagcta tatcacctag aacattgaat gcatgggtaa aagtaataga ggagaaggct
ttcaaccag aggtgatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacaa tgtaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc agaattgggag aggcacacac cagtgcagtc agggcctgtt
gcaccaggcc agatgagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa tagcatggat gacaagtaat ccacctattc cagtagggga catctataaa
agatggataa ttctgggggtt aaataaaaata gtaagaatgt atagccctgt tagcattttg
gacataaaaac aagggccaaa agaacccttt agagattatg tagatcggtt ctttaaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggcttcatta
gaagaaatga tgacagcatg tcagggagtg ggaggaccta gccataaagc aagggtgttg
gctgaggcaa tgagccaaac aaacagtaac atactagtgc agagaagcaa ttttaaaggc
tctaacagaa ttgttaaag tttcaactgt ggcaagggtg ggcacatagt cagaaattgc
agggccccta ggaaaaaggg ctgttggaag tgtggacagg aagggcacca aatgaaagac
tgtactgaga gacaggctaa ttttttaggg aaaatctggc cttcccacaa ggggaggcca
gggaatttcc tccagaacag accagagcca acagccccac cagcagaacc aacagcccca
ccagcagaga gcttcagggt cgaggagaca acccccgtgc cgaagaggga gaaagagagg
gaacctttaa cttccctcaa atcactcttt ggcaacgacc cctcgtcaca ataa

FIGURE 81 (SEQ ID NO:84)

atgggtgcga gagcgtcagt attgaaaggg aaaaaattag atacatggga aagaattagg
ttaaggccag ggggaaagaa acactatatg ctaaaacacc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagaaacag cagaaggctg taaacaaata
atgcaacagc tacaatcagc tcttcagaca ggaacagagg aacttagatc attatataac
acagtagcaa ctctctattg tgtacataaa gagatagatg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaataag agtcagcaaa aaacacagca agcagaagcg
gctgacaaag gaaaggtcag tcaaaattat ccaatagtgc agaactctca agggcaaag
gtacaccagg ccataatcacc gagaacttta aatgcatggg taaaagtaat agaagagaag
gctttcagcc cagaggtaat acccatgttt acagcattat cagaaggagc taccacacaa
gatttaaaca ccatgttaaa tacagtgggg ggacaccaag cagccatgca aatgttaaaa
gataccatca atgaggaggc tgcagaatgg gataggttac atccagtgca tgcagggcct
attgcaccag gccaaatgag agaaccaagg ggaagtgaca tagcaggaac tactagtacc
cttcaagaac aaatagcatg gatgacaagt aaccaccta ttccggtggg agacatctat
aaaagatgga taattctggg gttaaataaa atagtaagaa tgtatagccc tgtcagcatt
ttggacataa aacaagggcc aaaagaacct ttagagact atgtagaccg attctttaa
actttaaggg ctgaacaatc ttcacaagag gtaaaaaatt ggatgacaga cacttggtg
gtccaaaatg caaaccaga ttgtaagacc attttaagag cattaggacc aggggctaca
ttagaagaaa tgatgacagc atgtcaggga gtgggaggac ctggccacaa agcaagagtt
ttggctgagg caatgagcca agcaaataca aacataatga tgcagaaaag caattttaa
ggccctaaaa gaactgttaa atgtttcaat tgtggcaagg aagggcata agccagaaat
tgcagggcc ctaggaaaaa gggctgttg aaatgtggaa aggaaggaca ccaaatgaa
gactgtactg aaaggcaggc taattttta gggaaaattt ggccttccta caaggggagg
tcggggaatt tccttcagag cagaccagag ccatacagct caccagcaga gagcttcagg
ttcgaggagc gggagccgaa agacaaggaa ccacccttaa ctccctcaa atcactcttt
ggcagcgacc cctcgtcaca ataa

FIGURE 82 (SEQ ID NO:85)

atgggtgcga gagcgtcaat attaagaggg ggaaaattag ataaatggga aaaaattagg
ttaaggccag ggggaaagaa acgctatatg ataaaacacc tagtatgggc aagcagagag
ctggaaaaat tcgcacttaa ccctggcctt ttagagacat cagaaggatg taaacagata
atgaaacagc tacaaccagc tcttcagaca ggaacagagg aacttagatc attattcaac
accatagcag ttctctattg tgtacatgaa aagatagagg tacaagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa agtcagcaaa aaacacagca ggcagcagca
gctgacggaa aagtcagtca aaattatcct atagtgcaga atgccaagg gcaaatggtg
caccagagca tatcacctag gactttgaat gcatgggtaa aagtaataga ggagaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac ctcacaagac
ttaaacacca tgctaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc agaatgggat agaatacatc cagtacatgc ggggcctatt
gcaccaggcc aaatgagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa tagcatggat gacaagtaat ccacctatcc cagtgggaga catctataaa
agatggataa ttttggggtt aaataaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aagggccaaa ggaacccttt agagactatg tagacagggt ctttaaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagaaac cttgttggtc
caaaatgcaa acccagattg taagaccatt ttaagagggg taggaacagg ggctacatta
gagggaatga tgacagcatg tcagggagtg ggaggacctg gccataaagc aagagtgtta
gctgaagcaa tgagccaagc aacatataac ataatgatgc agagaagcaa ttttaaaggc
tctagaaaaa ttgttaaatg tttcaactgt ggcaggaaag ggcacatagc cagaaattgc
agggccccta gaaaaaaggg ctgttggaaa tgtggaaagg aaggacacca aatgagagaa
tgtactgaaa agcaggctaa ttttttaggg aaaatttggt cttcccacaa ggggaggcca
gggaatttcc ttcagagcag accagagcca acagccccc cagcagagag cttcagggtc
gaggagacac ccccgcgat gaagcaggaa ccgaaagaca gggaaccctt aactccctc
aaatcactct ttggcagcga cccctcgtca caataa

FIGURE 83 (SEQ ID NO:86)

```
atgggtgcga gagcgtcaat attaagaggg ggaaaattag ataatggga aaaaattagg
ttaaggccag ggggaaagaa acattatatg ataaaacacc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacag cagagggctg taaacaaata
ataaaacagc tacatccagc tcttcagaca ggaacagagg aacttagatc attatacaac
accgtggtaa ctctttattg cgtacatgca gagatagagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga aaaaaacaaa agtcagcaaa aaacacagca ggcaaaagcg
gctgacggaa aagtcagtca aaattatcct atagtacaga atctccaagg gcgaatggta
caccaagcca tatcacctag aaccttgaat gcatgggtaa aagtaataga ggaaaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac ccccaagac
ttaaacacca tgtaaatac agtgggggga catcaagcag ccatgcaa atgttaaagat
accatcaacg aggaggctgc agaattgggat agattacatc cagcacaggc agggcctgtt
gcaccaggcc aaataagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa taacatggat gacaagtaac ccacctgttc cagtgggaga aatctataaa
agatggataa ttctgggggtt aaataaaata gtaaggatgt atagccctgt cagcattttg
gacataaaac aagggccaaa ggaacccttt agagactatg tagaccggtt ctttaaaact
ttaagagctg aacaggctac acaagaagta aaaggctgga tgacagacac cttattggtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggctacacta
gaagaaatga tgacagcatg tcagggagtg ggaggaccta gccacaaggc aagagtgttg
gctgaggcaa tgagccaaac aaacagtgc agcataatga tgcagaaaag caattttaaa
ggagccaaaa gaattgttaa atgcttcaac tgtggcaagg aggggcacat agccagaaat
tgcagggccc ctaggaaaaa aggtgtttgg aaatgtggac aggaaggaca ccaaatgaaa
gactgtactg agaggcaggc taatttttta gggaaaattt ggccttccca caaaggaagg
ccagggaatt tccttcagaa cagaccagag ccaacagcac caccagcaga gagcttcagg
ttcagaggaga caacaccac tccgaagcag gagccgaagg acaggaacc ttaacttcc
ctcaaatcac tctttggcag cgaccctctg tcacaataa
```

FIGURE 84 (SEQ ID NO:87)

```
atgggtgcga gagcgtcaat attaagaggg ggaaaattag ataatggga aaaaattagg
ttaaggccag ggggaaagaa acattatatg ataaaacacc tagtatgggc aagcagggag
ctggaagat ttgcacttaa ccctggcctt ttagagacag cagagggctg taaacaaata
ataaacagc tacatccagc tcttcagaca ggaacagagg aacttagatc attatataac
accgtggcaa ctctttattg cgtacatgca gagatagagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa agtcagcaaa aaacacagca ggcaaaagcg
gctgacggaa aagtcagtca aaattatcct atagtacaga atctccaagg gcaaatggta
caccaggcca tatcacctag aaccttgaat gcatgggtaa aagtaataga ggaaaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac cccccaagac
ttaaacacca tgtaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaacg aggaggctgc agaatgggat agattacatc cagcacaggc agggcctgtt
gcaccaggcc aaataagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa taacatggat gacaagtaac ccacctgttc cagtgggaga aatctataaa
agatggataa ttctggggtt aaataaaata gtaaggatgt atagccctgt cagcattttg
gacataaaac aaggggccaaa ggaacccttt agagactatg tagaccggtt ctttaaaact
ttaagagctg aacaggctac acaagaagta aaaggctgga tgacagacac cttattggtc
caaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggctacacta
gaagaaatga tgacagcatg tcagggagtg ggaggacctt gccacaaggc aagagtgttg
gctgaggcaa tgagccaaac aaacagtgca agcataatga tgcagaaaag caatttttaa
ggagccaaaa gaattgttaa atgcttcaac tgtggcaagg aggggcacat agccagaaat
tgaggggccc ctaggaaaaa aggctgttgg aaatgtggac aggaaggaca ccaaatgaaa
gactgtactg agagacaggc taatttttta gggaaaattt ggccttccca caaaggaagg
ccagggaatt tccttcagaa cagaccagag tcaacagcac caccagcaga gagcttcagg
ttcgaggaga caacacccac tccgaagcag gagccgaagg acagggaacc ttagcttcc
ctcaaatcac tctttggcag cgaccctcg tcacaataa
```


FIGURE 85 (SEQ ID NO:88)

```
atgggtgcga gcg tcaatat taaaagggg aaaattagat gcatgggaaa gaattaggtt
aaggccaggg ggaaagaaac actatatgat aaaacattta gtatgggcaa gcaggagct
ggaaagattt gcacttaacc ctggcctgtt agagacatca gaaggatgta aacaaataat
gaaccagcta caaccatctc ttcagacagg aacagaagaa cttagatcat tatacaacac
agtagcaact ctctattgtg tacatgaaaa gatagaggta cgagacacca aggaagcctt
agacaagata gaggaagaac aaaacaaaag ccagcaaaaa acacaacagg caaaagcggc
tggcgaaaag gtcagtcaaa attatcctat agtgcagaat gcccaggggc aaatggtaca
ccaagctata tcacctagaa cgttaaatgc atgggtaaaa gtaatagagg agaaggcttt
cagcccagag gtaataccca tgtttacagc attatcagaa ggagccacc cacaagattt
aaacaccatg ttaaatacag tgggaggaca tcaagcagcc atgcaaagt taaaagatac
catcaatgag gaagctgcag aatgggatag ggtacatcca gtgcatgcag ggcctgttgc
accaggacag atgagagaac caaggggaag tgacatagca ggaactacta gtaccctgca
ggaacaaata gcatggatga caagtaatcc acctattcca gtaggagaaa ttataaaaag
atggataatt ctgggggttaa ataaaatagt aagaatgtat agccctgtca gcatcttggg
cataaaacaa gggccaaagg aacccttttag ggactatgta gaccggttct ttaaaacttt
aagagccgaa caggctacac aagatgtaaa aaattggatg acagacacct tgttggtcca
aaatgcgaac ccagattgta agaccatttt aagagcatta ggaccagggg cttcattaga
agaaatgatg acagcatgtc agggagtggg aggacctagc cacaaagcaa gagtggtggc
tgaggcaatg agccaagcaa acaatataaa catactgatg cagagaagca attttaaggg
ctctaagaga attgttaa atgttcaactg tggcaaggaa gggcacatag ccagaaattg
cagggccctt aggaaaaagg gctgttgga atgtggaaag gaaggacacc aaataaaaga
ctgtactgag aggcaggcta attttttagg gaaaatttgg ccttcccgcagg ggggaggcc
aggggaatttc cttcagaaca ggccagagcc aacagcccca ccagcagaaa gcttcaggtt
cgaggagaca acccctgcgc cgaagcagga caaggaacc ttaacttccc tcaaatcact
ctttggcagc gaccctcgt cacaataa
```

FIGURE 86 (SEQ ID NO:89)

atgggtgcga gagcgtcaac attaaaaggg ggaaaattag atgcatggga aagaattagg
ttaaggccag ggggaaagaa acactatatg ataaaacatt tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctg ttagagacat cagaaggatg taaacaaata
atgaaccagc tacaaccatc tcttcagaca ggaacagaag aacttagatc attatacaac
acagtagcaa ctctctattg tgtacatgaa aagatagagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa agccagcaaa aaacacaaca ggcaaaggcg
gctggcgaaa aggtcagtca aaattatcct atagtgcaga atgcccagg gcaaatggta
caccaagcta tatgcctag aacgttaaata gcatgggtaa aagtaataga ggagaaggct
ttcagcccag aggtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgttaaatac agtgggagga catcaagcag ctatgcaaat gttaaagat
accatcaatg aggaagctgc agaatgggat aggggtacac cagtgcacgc aaggcctgtt
gcaccaggac agatgagaga accaagggga agtgacatag caggaaactac tagtaccctg
caggaacaaa tagcatggat gacaagtaat ccacctatc cagtaggaga aatttataaa
agatggataa ttctgggggtt aaataaaaata gtaagaatgt atagccctgt cagcatcttg
gacataaaac aaggggccaaa ggaacccttt agggactatg tagaccgggtt cttttaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagaccatt ttaagagcat tagggccagg ggcttcatta
gaagaaatga taacagcatg tcagggagtg ggaggaccta gccacaaaagc aagagtgttg
gctgaggcaa tgagccaagc aaacaatata aacatactga tgcagagaag caattttaag
ggctctaaga gaattgttaa atgcttcaac tgtggcaagg aagggcacat agccaaaaat
tgcagagccc ctaggaaaaa gggctgttga aaatgtagaa aagaaagaca ccaaatgaaa
gactgtactg aaaggcaggc taatttttta gggaaaattt ggccttccca caaggggagg
ccagggaatt tccttcagaa caggccagag ccaacagccc caccagcaga aagcttcagg
ttcgagaaga caaccctgc gccgaagcag gacaaggaac ccttaacttc cctcaaatca
ctctttggca gcgaccctc gtcacaataa

FIGURE 87 (SEQ ID NO:90)

```
atgggtgcga gagcgtcaat attaagaggg ggaaaattag ataaatggga agaaattagg
ttaaggccag ggggaaagaa aacctatagg ctaaaacatc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacag cagaaggctg taaacaaata
ataagacagc tacaccagc tcttcagaca ggaacggagg aacttagatc attatacaac
acagtagcaa ctctctattg tgtacatgca aacatagagg taaaagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa agtcagcaaa aatcagagca ggcaaaagta
ggtaacgaaa agatcagtca aaattatcct atagtgcaga atctccaagg gcaaatggta
caccaggcct tatcacctag aactttgaat gcatgggtaa aagtaataga ggagaaggct
ttcagcccag aggtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgtaaacac agtggggggg catcaagcag ccatgcaaat gttaaaagac
accatcaatg aagaggctgc agaatgggat cgattacacc cagtacatgc agggcctatt
gcaccaggcc aaatgagaga accaagggga agtgacatag caggaactac tagcaccctt
caggaacaaa tagcatggat gacaagtaac ccacctattc cgggtgggaga tatctataaa
agatggataa ttctgggggt aaataaaata gtaagaatgt atagccctgt cagcattttg
gacattaaac aagggccaaa ggaacccttt agagactatg tagaccggtt ctttaaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagatcatt ttaagaggat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcagggagtg ggaggaccta gccacaaagc aagagtgttg
gctgaggcaa tgagccaagc aaacagtgga aacataatga tgcagaaaag caattttaga
ggctctaaaa gaattattaa atgttttaac tgtggcaagg aagggcacat agccaaaaat
tgtaaggccc ctaggaaaag aggctgttgg aaatgtggaa aggaaggaca ccaaatgaaa
gactgtactg aaagacaggc taatttttta gggaaaattt ggcttcctg caaggggagg
ccaggggaatt tccttcagaa caggccagag ccaacagccc caccagcaga gccaacagcc
ccaccagcag agagcctcag gatcgaggaa acaacccccg ctccgaagcc ggagccgagg
gacaggggaac ccttaatctc cctcaaatca ccctttggca gcgaccctc gtcacaataa
```

FIGURE 88 (SEQ ID NO:91)

atgggtgcga gagcgtcagt attaagagggc gaaaaattag atacatggga aaaaattagg
ttaaggccag ggggaaagaa acgctatatg ctaaaacaca tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacat cagaaggctg taaacaaata
atacaacagc tacaaccagc tcttcagaca ggaacagagg aacttaaatc gttattcaac
acagtagcaa ctctctattg tgtacataaa aagatagagg ttcgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa agtcagcaaa aaacacagca ggcagaagcg
gctgacaaaa aggtcagtc aaattatcct atagtacaga acctccaagg gcaaatggta
caccaagccc tatcacctag aactttgaat gcatgggtaa aagtaataga ggagaaggct
tttggcccag aggttaatacc catgtttaca gcattatcag aaggagccac ccagcagat
ttaaacacca tgttaataac agtgggggga catcaggcag ccatgcagat gttaaaagat
accatcaatg aggaggctgc agaatgggac agattacacc cagtacatgc agggcctact
gcaccaggcc aaatgagaga acctagggga agtgacatag caggaactac tagtaccctt
caggaacaaa tagctcggat gacaagtaac ccacctgtcc cagtgggaga catctataaa
agatggataa ttctaggggt aaataaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac agggggccaa agaacccttt agagactatg tagaccgggt ctttaaaact
ttaagagctg aacaagctac acaagaggta aaaggttgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcaggagtg ggaggacctg gccacaaagc cagagtgttg
gctgaggcaa tgagccaagc aaacagtaac atacttatgc agagaagcaa ttttaaggc
tctaaaagaa ttgttaaatg tttcaactgt ggcaaggaag ggcacatagc cggaaattgc
agggccccta gaaaaaaggg ctgttggaag tgtggaaaag aaggacacca aatgaaagaa
tgtactgaaa ggcaggctaa ttttttaggg aaaatttggc cttcccacaa ggggaggcca
gggaatttcc tccagagcag accagagcca acagcccac cagcagagag cttcaggttc
gaggagacaa cccccgtcc gaagcaggag tcgaaagaca gggagccctt aacttccttc
agatcactct ttggcaacga ccctcgtca caataa

FIGURE 89 (SEQ ID NO:92)

atgggtgcga gagcgtcagt attaagaggc gaaaaattgg atacatggga aaagattagg
ttaaggccag ggggaaagaa acgctatatg ctaaaacaca tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacat cagaaggctg taaacaaata
atacaacagc tacaaccagc tcttcagaca ggaacagagg aacttaaate attattcaac
acagtagcaa ctctctattg tgtacacaga aagatagagg tacgagacac caaagaagcc
ttagacaaga tagaggaaga acgaaacaaa agtcagcaaa aaacacagca ggcagaagcg
gctgacaaaa aggtcagtca aaattatcct atagtacaga atctccaagg gcaaatggta
caccaggccc tatcacctag aactttgaat gcatgggtaa aagtaataga ggagaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac ccagcagat
ttaaacacca tgtaaatac agtgggggga catcaagcag ccatgcagat gttaaaagat
accatcaatg aggaggctgc agaattgggac agattacacc cagtacatgc agggcctgct
gcaccaggcc aatgagaga acctagggga agtgacatag caggaaactac tagtaccctt
caggaacaaa tagcatggat gacaagtaac ccacctgtcc cagtgggaga catctataaa
agatggataa ttctagggtt aaataaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aggggccaaa agaacccttt agagactatg tagaccggtt ctttaaaact
ttaagagctg aacaagctac acaagaggta aaagggtgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcaggaggatg ggaggacctg gccacaaagc cagagtattg
gctgaggcaa tgagccaagc aaacagtaac atattttatgc agagaagcaa ttttaaaggc
tctaaaagaa ttgttaaattg tttcaactgt ggcaaggaag ggcacatagc caaaaattgc
agggccccta gaaaaaaggg ctgttggaag tgtggaaaag aaggacacca aatgaaagac
tgtactgaaa ggcaggctaa ttttttaggg aaaatttggc cttccacaa ggggaggcca
gggaatttcc tccagagcag accagagcca acagccccac cagcagagaa cttcaggttc
gaggagacaa cccccgctcc gaagcaggag tcgaaagaca gggagccctt aacttcctc
agatcactct ttggcaacga cccctcgtca caataa

FIGURE 90 (SEQ ID NO:93)

atgggtgcga gagcgtcaat attaagagggc ggaaaattag ataaatggga aaaaattaga
ttaaggccag ggggaaagaa acactatatg ttaaaacaca tagtatgggc aagcagggag
ctggaaagat ttgcaactta ccctggcctt ttagagacat cagaaggctg taaacaaata
atacaacagc tacacacagc tcttaagaca ggaacagagg aacttacatc attatacaac
acagtagcaa ctctctactg tgtacatgca gggatagagg tacgagacac caaggaggcc
ttagacaaga tagaggagga gcaaaacaaa agtcagaaaa aaatgcagca agcagaagtg
gctgacaaaa agaaggtcag tcaaaattat cctatagtac agaatacca agggcaaatg
gtacaccaga acatatcacc aagaacttta aatgcatggg taaaagtaat agaggagaag
ggtttcaacc cagaggtaat acccatgttt acagcattat cagagggagc cacccttct
gatctgaaca ccatgttaaa tatagtgggg ggacatcaag cagccatgca aatgttaaaa
gataccatca atgaggaggc tgcagaatgg gatagattac acccagcaca ggcagggcct
gttgaccag gccaaatcag agatccaagg ggaagtgaca tagcaggaac tactagtacc
cttcaggaac aagtaacatg gatgacaaat aaccaccta ttccagtagg agacatctat
aaaagatgga taattctggg attaaataaa atagtaagaa tgtatagccc tgtcagcatt
ttggacatta gacaaggacc aaaggagcct tttagagact atgtagatcg gttctttaaa
actttaagag ctgaacaagc tacacaagat gtaaaaaatt ggatgacaga caccttggtg
gtccaaaatg caaaccaga ttgtaagacc attttaagag cattaggacc aggggctaca
ttagaagaaa tgatgacagc atgtcaagga gtgggaggac ctagccacaa agcaagagtc
ttggctgagg caatgagcca agcaggcaat acaaacataa tgatgcagaa aagcaatttc
aaaggcccta gaagaactat taaatgcttc aactgtggca aggaaggaca cctagccaga
aattgcaggg cccctaggaa aaaaggctgt tggaaatgtg gaaaggaagg acaccaaag
aaagactgta ctgagaggca ggctaatttt ttagggaaaa tttggccttc ccactcgggg
aggccaggga acttccttca gaacagacca gagccaacag cccaccagc agagagcttc
aggttcgagg agacaacccc cgctcagaag caggagccgc aagacagga acccttaact
cccctcaaat cactctttgg cggcgacccc tcgtcacaat aa

FIGURE 91 (SEQ ID NO:94)

```
atgggtgcga gagcgtcaat attaagaggg ggaaaattag ataaatggga aaaaattagg
ttaaggccag gggggaaaaa acactatatg ctaaaacacc tagtatgggc aagcagagag
ctggaaagat ttgcagttaa ccctggcctt ttagagacat cagacggatg tagacaaata
ataaaacagc tacaaccagc tcttcagaca ggaacagagg aaattagatc attatttaac
acagtagcaa ctctctattg tgtacatgaa gggatagatg tacgagacac caaggaagcc
ttagacaagt tggaggagga acaaaacaaa tgtcagcaaa aaacacagca ggcagaagcg
gctgacaaaa aggtcagtca aaattatcct atagtgcaga acctccaagg gcaaattgta
caccaggcca tatcacctag aaccttgaat gcatgggtaa aagtaataga ggagaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac ccacaagat
ttaaacacca tgttaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc cgaatgggat aggttacatc cagtacatgc agggcctgtt
gcaccaggcc agatgagaga accaagggga agtgacatag cagaaactac tagtaccctt
caagaacaaa tagcatggat gacaagtaac ccacctatcc cagtaggaga catctataaa
aggtggataa ttctgggggt aaataaaata gtaagaatgt acagccctgt cagcattttg
gacataaaac aaggaccaa ggaacccttt agagactatg tagaccggtt cttcaaaact
ttaagagctg aacaatctac acaagaggta aaaaattgga tgacagacac cttgttagtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggcttcatta
gaagaaatga tgacagcatg tcagggagtg ggaggacctt gccacaaagc aagagctttg
gctgaggcaa tgagccaagc aaacaatgca agtgtaatga tgcagaaaag caattttaaa
ggccctagaa gtactgttaa atgtttcaac tgtggcaagg aagggcacat agccaggaaat
tgcagggccc ctaggaaaaa ggactgttgg aaatgtggaa aggaaggaca ccaaatgaaa
gactgtactg agagacaggc taatttttta gggaaaattt ggccttccca caaggggagg
ccagggaatt tccttcagag caggccagag ccaacagccc caccactaga gccaacagcc
ccaccagcag agagcttcaa gttcgaggag actccgaagc gggagccgaa agacagggaa
cccttaactt ccctcaaate actctttggc agcgaccctt cgtcacaata a
```

FIGURE 92 (SEQ ID NO:95)

atgggtgcga gagcgtcaat attaagaggg ggaaaattag acaaatggga aaaaattagg
ttaaggccag gggggaaaaa acgctatatg ctaaaacacc tagtatgggc aagcagagag
ctggacagat ttgcagttaa ccctggcctt ttagagacat cagacggatg tagacaaata
ataaaacagc tacaaccagc tcttcagaca ggaacagagg aaattagatc attatttaac
acagtagcaa ctctctattg tgtacataaa gggatagatg tacgagacac caaggaagcc
ttagacaaga tagaggagga acaaaacaaa tgccagcaaa aaacacagca ggcggaagcg
gctgacaaaa aggtcagtca aaattatcct atagtgcaga acctccaagg gcaaatggta
caccaggcca tatcacctag aaccttgaat gcatgggtaa aagtaataga ggagaaggct
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgttaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc cgaatgggat aggttacatc cagtacatgc agggcctgtt
gcaccaggcc agatgagaga accaagggga agtgacatag cagaaactac tagtaccctt
caagaacaaa tagcatggat gacaagtaac ccacctatcc cagtaggaga catctataaa
aggtggataa ttctgggggt aaataaaata gtaagaatgt acagccctgt cagcattttg
gacataaaac aaggaccaa aagaaccttt agagactatg tagaccggtt cttcaaaact
ttaagagctg aacaatctac acaagaggta aaaaattgga tgacagacac cttgttagtc
caaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggcttcatta
gaagaaatga tgacagcatg tcagggagtg ggaggacctt cccacaaagc aagagttttg
gctgaggcaa tgagccaagc aaacaataca agtgtaatga tacagaaaag caatttttaa
ggccctagaa gagctgttaa atgtttcaac tgtggcaagg aagggcacat agccaggaat
tgcaagggccc ctaggaaaaa gggctgttgg aaatgtggaa aggaaggaca ccaaatgaaa
gactgtactg agagacaggc taatttttta gggaaaattt ggccttccca caaggaagg
ccagggaatt tccttcagag cagaccagag ccaacagccc caccactaga accaacagcc
ccaccagcag agagcttcaa gttcgaggag actccgaagc aggagccgaa agacagggaa
ccctacaggg aacccttaac ttccctcaaa tcactctttg gcagcgacce ctcgtcacia
taa

FIGURE 93 (SEQ ID NO:96)

```
atgggtgcga gagcgtcaat attaagaggg acgaaattag atgcatggga aaaaattagg
ttaaggccag ggggaaagaa acattatatg ttaaaacacc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagaaacat cggaaggctg taaacaaata
atgaaacagc tacaccagc tcttcagaca ggaacagagg aacttaaadc attatacaac
acagtagcaa ctctctattg tgtacatgaa agcataaagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa attaaaagtc agcaaaaaac acagcaggca
aaagcggctg acgaaaaagt cagtcaaaat tatcctatag tgcagaatct tcaagggcaa
atggtacatc agaacctatc acctagaacc ttgaatgcat gggtaaaagt aatagaggag
aaggctttta gccagaggt aatacccatg tttacagcat tatcagaagg agccaccca
caagatttaa acaccatgtt aaatacgggt gggggacatc aagcagccat gcaaatgtta
aaagatccca tcaatgaaga ggctgcagaa tgggatagat tacaccagc ccatgcgggg
cctatggcac caggccaatt gagagaacca aggggaagtg acatagcagg aactactagt
acccttcagg aacaaatagc atggatgaca agtaatccac ctatcccagt gggagacatc
tataaaagat ggataattct ggggttaaata aaaatagtga gaatgtatag ccctatcagc
atthttggaca taagacaagg gccaaaggaa ccttttagag actatgtaga ccggttcttt
aaagccttaa gagctgaaca agctacacaa gatgtaaaaa attggatgac agaaaccttg
ctggtccaaa atgcgaaccc agattgtatg accattttta aagcattagg aataggggct
acattggaag aaatgatgac agcatgtcag ggagtggggg gacctagtca caaagcaaga
gtgttagctg aggcaatgag ccaagcaaac aatacaaca taatgatgca gagaagcaat
tttaaaagct caaaaagaat tgtaaataatg ttcaactgtg gcaaggaagg gcataatagcc
agaaattgca gggcccctag gaaaaagggc tgttggaaat gtggaaagga aggacaccaa
atgaaagatt gtactgagag gcaggcaaat ttttttagga aaatttgcc tccccacaag
gggaggccag ggaatttcct tcagaacaga ccagagccaa cagccccacc agcagagagt
ttcaggaaca gaccagagcc aacggctcca ccagcagaga gcttcagggt cgaggagaca
acccccactc cgaagcagga gccgaaagac agggatccct taacttccct caaatcactc
tttggcagcg acccctcgtc acaataa
```

FIGURE 94 (SEQ ID NO:97)

```
atgggtgcga gagcgtcaat attaagaggg acgaaattag atgcatggga aaaaattagg
ttaaggccag ggggaaagaa acattatatg ttaaaacacc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagaaacat cagaaggctg taaacaaata
atgaaacagc tacacccagc tcttcagaca ggaacagagg aacttaaate attatacaac
acagtagcaa ctctctattg tgtacatgaa aacataaagg tacgagacac caaggaagcc
ttagacaaga tagaggaaga acaaaacaaa attaaaagtc agcaaaaaac acagcaggca
aaagcggctg acgaaaagt cagtcaaaat tatectatag tgcagaatct tcaagggcaa
atggtacatc agaacctatc acctagaacc ttgaatgcat gggtaaaagt aatagaggag
aaggctttta gccagagggt aatacccatg ttacagcat tatcagaagg agccaccca
caagatttaa gcaccatgtt aaatacgggtg gggggacatc aagcagccat gcaaatgtta
aaagatacca tcaatgaaga ggctgcagaa tgggatagat tacaccagt ccatgcgggg
cctatggcac caggccaatt gagagaacca aggggaagt acatagcagg aactactagt
acccttcggg aacaaatagc atggatgaca agtaatccac ctatcccagt gggagacatc
tataaaagat ggataattct ggggttaaata aaaatagtga gaatgtatag ccctgtcagc
atgttgagca taagacaagg gccaaaggaa ccctttagag actatgtaga ccggttcttt
aaagccttaa gagctgaaca agctacacaa gatgtaaaaa attggatgac agaaaccttg
ctggtccaaa atgcgaacct agattgtaag accattttta aagcattagg aataggggct
acattggaag aaatgatgac agcatgtcag ggagtggggg gacctagtca caaagcaaga
gtgttagctg aggcaatgag ccaagcaaac aatacaaaaca taatgatgca gagaagcaat
tttaaaagct caaaaagaat tgtaaatgt tccaactgtg gcaaggaagg gcatatagcc
agaaattgca gggcccctag gaaaaagggc tgttggaat gtggaaagga aggacaccaa
atgaaagatt gtactgagag gcaggcaaat tttttaggga aaatttggcc ttcccacaag
gggaggccag ggaatttcct tcagaacaga ccagagccaa cagccccacc agcagagagt
ttcaggaaca gaccagagcc aacggctcca ccagcagaga gcttcagggt cgaggagaca
acccccactc cgaagcagga gccgaaagac agggatccct taacttcct caaatcactc
tttggpaggc acccctcgtc acaataa
```

FIGURE 95 (SEQ ID NO:98)

atgggtgcga gagcgtcaat attaagaggg gaaaaattag ataaatggga gaaaattagg
ctaaggccag ggggaaggaa acactatatg ctaaaacatc tagtatgggc aagcagagag
ctggaagat tcgcacttaa ccctggcctt ttagagacat cacaaggctg taaacaaata
ataaaacagc tacaccacagc tcttaagaca ggaacagagg aacttaggtc attatacaac
acagtagcaa ctctctattg tgtacatgaa aacatagagg tacgagacac caaggaggcc
ttagacaaga tagaggaaga acaaaacaaa agtcagcaaa aaacacagca ggcaaaagcg
gctgacgaag gagtcagtca aaattatccc atagtgcaga atctccaagg gcaaatggta
caccaggcca tatcacctag aactttgaat gcatgggtga aagtaataga ggagaaggct
tttagcccag aagtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgttaaatac agtaggggga catcaagcag ccatgcagat gttaaaagat
accatcaatg aggaggctgc agaatgggat agattacatc cagtccatgc agggcctgct
gcaccaggcc aatgagggga acctagagga agtgacatag caggaactac tagtacctt
caggaacaaa tagcatggat gacaggtaac ccacctgtcc cagtgggaga catctataaa
agatggataa ttctggggtt aaataaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aaggggccaaa ggaacccttt agagactatg tagatcggtt ctttaaagtt
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttgatc
caaatgcga acccagattg taagaccatc ttaaaggcat tgggaccagc ggcttcatta
gaagaaatga tgacagcatg tcaggagtg ggaggacctg gccacaaagc aagagtgttg
gctgaggcaa tgagccaagc aaacagtaac ataatgatgc agagaagcaa ttttaaagga
tctaaaagaa ttgttaaatg tttcaactgt ggcaaggaag ggcacatagc cagaaattgc
agggccccta gaaaaaaggg ctgttggaag tgtggacaag aaggacacca aatgaaagac
tgtactgaaa ggcaggctaa ttttttaggg aaaatttggc cttcccacaa ggggaggcca
gggaatttcc tccagagcag gccagagcca acagcccac cagcagagag cttcaggttc
gaggaacaaa ccccgctcc gaaacaggag tcgaaggaca gggaaccctt aatttcctc
aatcactct ttggcagcga ccctcgtca caataa

FIGURE 96 (SEQ ID NO:99)

atgggtgcga gagcgtcaat attaaaaggc gaaaaattag atagatggga aagaattagg
ttaaggccag ggggaaagaa acattatatg ttaaaacaca tagtatgggc aagcagggag
ttggaaaaat ttgcacttaa ccctggcctt ttagaaacag cagaaggctg taatcaaata
atgaaccagc tacaaccagc tcttcagaca ggaacagagg aacttaaate attattcaac
acagtagcaa ctctctattg tgtacataaa aagatagatg tacgagacac caaggaagcc
ttagataaga tagaggaaga acaaaacaaa agtcagcaaa aaacacagca ggcaaaagcg
gctgacgaaa aggtcagtca aaattatcct atagtacaaa atctccaagg gcaaatggta
catcaagcca tatcacctag aaccttgaat gcatgggtaa aagtaataga ggagaaggcc
tttagcccag aggtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgttaaatac ggtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc agaattggat agattacatc cagtacatgc ggggcctgtt
gcaccaggcc aaatgagaga accaagggga agtgacatag caggaactac tagtaccctt
caggaacaaa tagcatggat tacagctaac ccacctattc cagtaggaga aatctataaa
agatggataa ttctggggtt aaataaaaata gtgagaatgt atagccctgt cagcattttg
gacataagac aaggaccaaa ggaacccttt agagactatg tagatcggtt ctttaaaact
ttaagagctg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagaccatt ttaagagcat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcagggagtg ggaggacctt gccacaaagc aagagttttg
gctgaggcaa tgagccaagc aaacaatgca gtcataatga tgcagaaaag caatttttaa
ggctcctagaa aaattattag atgtttcaac tgtggttaagg aaggggacat agccagaaac
tgcagggccc ctaggaaaaa aggctgttgg aaatgtggaa aggagggaca ccaaataaaa
gactgtactg aaaggcaggc taatttttta gggaaaattt ggccttccca caaggggagg
ccaggggaatt tccttcagaa cagaccagag ccaacagccc caccagcaga gagcttcaag
ttcgaggaga caacccccac tccgaggcag gagtcgaaag acaggggaacc ctttaacttcc
ctcaaatcac tctttggcag cgaccctcgc tcacaataa

FIGURE 97 (SEQ ID NO:100)

```
atgggtgcga gagcgtcaat attaagaggc ggaaaattag atacatggga aaaaattagg
ttaaggccag ggggaaagaa acactatatg ctaaaacatc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacat cagaaggctg taaacaaata
ataagacagc tacaaccagc tcttcagaca ggaacagagg aacttaaadc attatataac
acagtagcaa ctctctattg tgtacatgca aagatagagg tacgagacac caaggagcc
ttagacagga tagaggaaga acagaaaaaa tgtcagcaaa aaacacagca ggcaaaagag
gctgacggga agatcagtca aaattatcct atagtgcaga atcttcaagg gcaaatggta
caccaggcca tatcacctag aactttgaat gcattatcag aaggagccac cccacaagat
tttagcccag aagtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgctaaatac agtgggggga catcaagcag ccatgcaaag gttaaaagat
accatcaatg aggaggctgc agaattgggac agaatacatc cagtacatgc agggcctatt
gcaccaggcc aaatgagaga accaagggga agtgacatag caggaaactac tagtacctt
caggaacaaa tagcatggat gacaagtaac ccacctgttc cagtgggaga aatctataaa
agatggataa ttctgggcct aaataaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aaggacccaaa ggaacccttt agagattatg tagatcggtt ctttaaaact
ttaagagccg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagatcatt ttaagaggat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcaggaggatg ggaggacctg gccacaaagc aagagtgttg
gctgaggcaa tgagccaagc aaacagtaca aatataatga tgcagagagg caattttaaa
ggccctaaaa gaaacattaa atgttttaac tgtggcaagg aagggcacct agccagaaat
tacaggggcc ctaggaaaaa aggttggttg aaatgtggaa aagaaggaca ccaaatgaaa
gactgtacag agagacaggc taatttttta gggaaaattt ggccttccca caagggaagg
ccagggaact tccttcagaa cagaacagag ccaacagccc caccagcaga gagcttcagg
ttcgaggaga caaacctgc tcgaagcag gagccgaaag acagggaacc ctttaacttcc
ctcaaatcac tctttggcag cgaccctcg tcacaataa
```

FIGURE 98 (SEQ ID NO:101)

atgggtgcga gagcgtcaat attaggagggc ggaaaattag atacatggga aaaaattagg
ttaaggccag ggggaaagaa acactatatg ctaaaacatc tagtatgggc aagcagggag
ctggaaagat ttgcacttaa ccctggcctt ttagagacat cagaaggctg taaacaaata
ataagacaac tacaaccagc tcttcagaca ggaacagagg aacttaaadc attatacaac
acagtagcaa ctctctattg tgtacatgca aagatagagg tacgagacac caaggaagcc
ttagataaga tagaggaaga acagaaaaaa tgtcagcaaa aaacacagca ggcaaaagag
gctgacggga agatcagtcg aaattatcct atagtgcaga atcttcaagg gcaaatggta
caccaggcca tatcacctag aactttgaat gcatgggtaa aagtaataga ggagaaggct
tttagcccag aagtaatacc catgtttaca gcattatcag aaggagccac cccacaagat
ttaaacacca tgctaaatac agtgggggga catcaagcag ccatgcaaat gttaaaagat
accatcaatg aggaggctgc agaatgggac agaatacatc cagtacatgc agggcctatt
gcaccaggcc aatgagaga accaagggga agtgacatag caggaaactac tagtaccctt
caggaacaaa tagcatggat gacaagtaac ccacctgttc cagtgggaga aatctataaa
agatggataa ttctgggctt aaataaaaata gtaagaatgt atagccctgt cagcattttg
gacataaaac aaggaccaa ggaacccttt agagattatg tagaccggtt ctttaaaact
ttaagagccg aacaagctac acaagatgta aaaaattgga tgacagacac cttgttggtc
caaaatgcga acccagattg taagatcatt ttaagaggat taggaccagg ggctacatta
gaagaaatga tgacagcatg tcaggaggag ggaggacctg gccacaaagc aagagtgttg
gctgaggcaa tgagccaagc aaacagtaca aatataatga tgcagagagg caatttttaa
ggccctaaaa gaaacattaa atgttttaac tgtggcaagg aagggcacct agccagaaat
tgcaggggcc ctaggaaaaa gggttgttgg aaatgtggaa aagaaggaca ccaaatgaaa
gactgtacag agagacaggc taatttttta gggaaaattt ggccttccca caagggaaga
ccagggaact tccttcagaa ccgaacagag ccaacagccc caccagcaga gagcttcagg
ttcgaggaga caaacctgc tccgaagcag gagccgaaag acagggaacc cttaacttcc
ctcaaatcac tctttggcag cgaccctcg tcacaataa

Figure 99a1: Nef

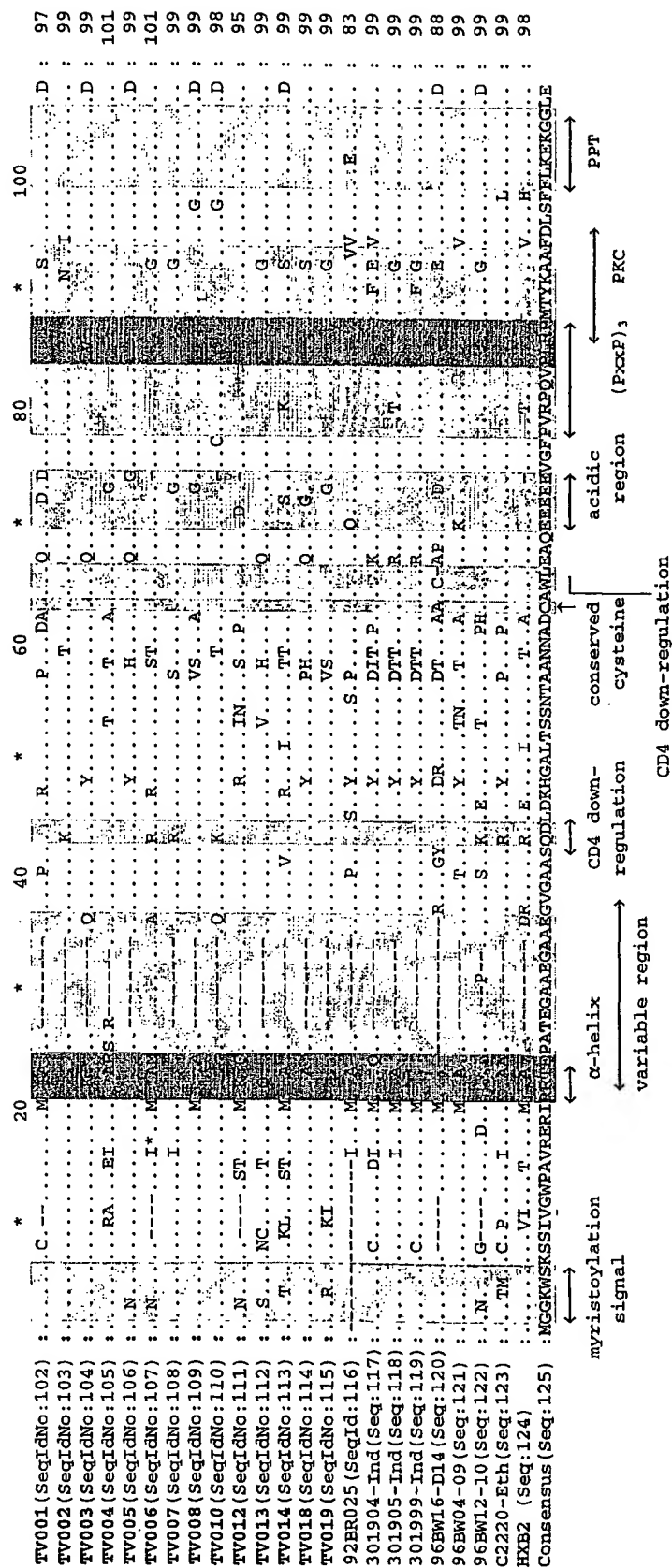


Figure 99a2: Nef (continued)

TV001 (seq:102)	: V	*	120	*	140	*	160	*	180	*	200	*	205
TV002 (seq:103)	:												207
TV003 (seq:104)	:												207
TV004 (seq:105)	:												209
TV005 (seq:106)	:												209
TV006 (seq:107)	:												207
TV007 (seq:108)	:												207
TV008 (seq:109)	:												207
TV010 (seq:110)	:												207
TV012 (seq:111)	:												203
TV013 (seq:112)	:												207
TV014 (seq:113)	:												207
TV018 (seq:114)	:												207
TV019 (seq:115)	:												191
92BR025 (seq:116)	:												207
301904-Ind (seq:117)	:												207
301905-Ind (seq:118)	:												207
301999-Ind (seq:119)	:												207
96BW16-D14 (seq:120)	:												196
96BW04-09 (seq:121)	:												207
96BW12-10 (seq:122)	:												207
C2220-8th (seq:123)	:												207
HXB2 (seq:124)	:												205
Consensus (seq:125)	: GLIYSKKEOEILDLWVYHTQGFPPDMQNTPGPGVRYPLTFGCWCFKLVFPDIPREVSEANEGENNCCLLHPMSQHGMEDEEDREVLKWKFDSSLARHMHARELHPEYKDC												

PAK binding ↔
 HXB2 premature stop ↑
 β-turn ↔
 PxxP ↔
 interaction with Aps - CD4 down-regulation ↔
 CD4 down-regulation ↔
 COP I

Figure 99c: Rev

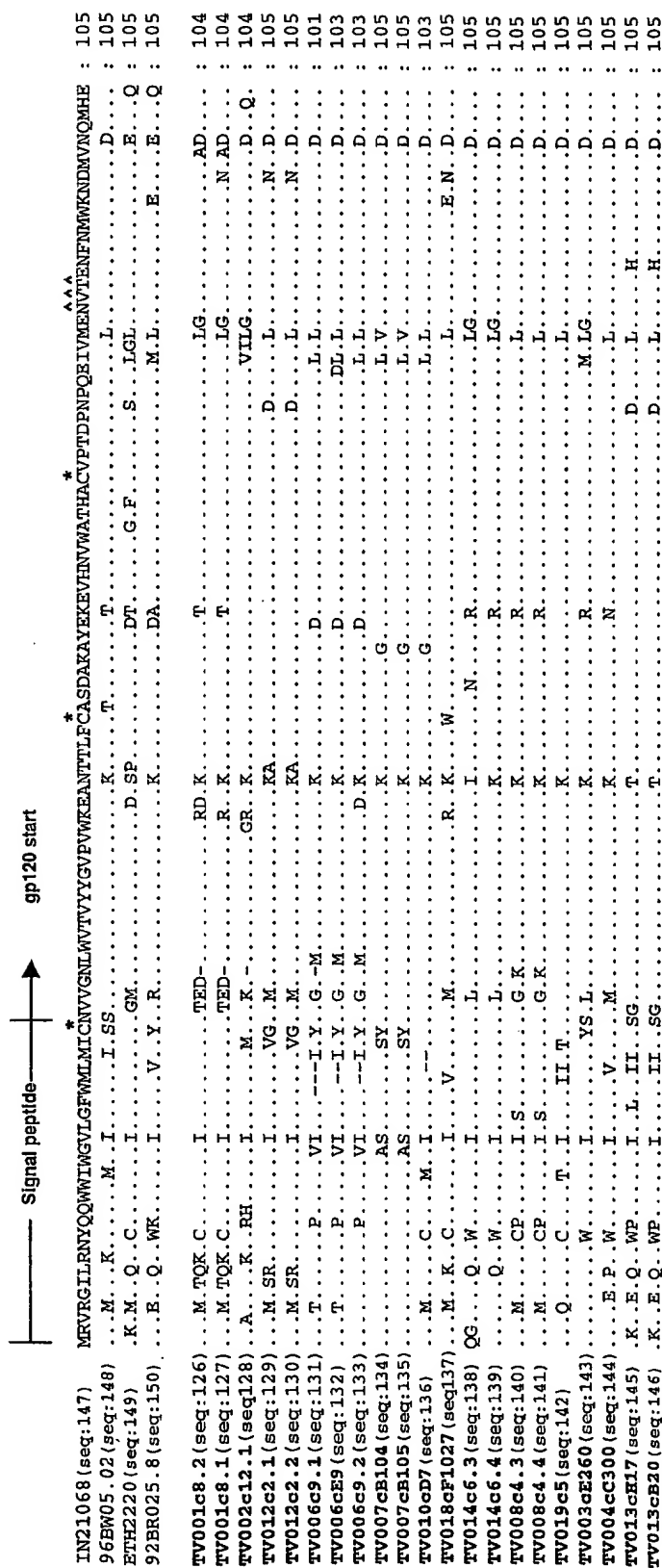
```

↑
subtype C
premature stop

```

activation
domain, NES

exon 1/exon 2 RNA binding multimerisation
multimerisation motif, NLS surface
surface



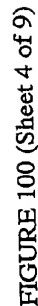
[illegible]

FIGURE 100 (Sheet 2 of 9)



IN21068	: SS---	GYTRLINCNTSALTQACPKVTFDPIPIHYCAPAGYAILKCNKNTFNGTGPCNVSTVQCTHGKPKVVTQLLNGSLAEGGIIRSENLTNNVKTIIVHL	: 294
96BW05.02	: N---NE	: I---S---T---Q---N---S---A---V---K---E---A---I---Q---	: 286
ETH2220	: ---FD	: TI---SL---RD---T---I---ET---F---A---I---Q---	: 285
92BR025.8	: T---D	: I---S---S---N---I---T---EE---K---D---D---	: 286
TV001c8.2	: FT---	: TI---S---Y---E---E---T---	: 295
TV001c8.1	: FT---	: TI---S---D---E---E---T---	: 295
TV002c12.1	: ---INN	: I---S---P---K---I---D---EE---	: 281
TV013c2.1	: ---K	: I---S---S---N---A---I---EE---V---M---A---I---	: 278
TV013c2.2	: ---K	: I---S---S---N---A---I---EE---V---M---A---I---	: 278
TV006c9.1	: ---DNS-G	: I---S---S---S---G---D---Q---A---I---	: 283
TV006cE9	: ---	: I---S---S---S---G---D---Q---A---I---	: 289
TV006c9.2	: ---	: I---S---S---S---G---D---Q---A---I---	: 285
TV007cB104	: ---T	: T---T---T---T---T---T---K---K---K---AQ---	: 288
TV007cB105	: ---T	: T---T---T---T---T---T---K---K---K---AQ---	: 288
TV010cD7	: ---E	: TI---S---S---I---E---M---A---F---	: 277
TV019cF1027	: ---E	: I---S---S---N---E---K---M---	: 290
TV014c6.3	: ---EK	: T---S---T---E---E---A---I---Q---	: 294
TV014c6.4	: ---EK	: T---S---T---E---E---A---I---Q---	: 294
TV008c4.3	: K---TEC	: TV---S---E---N---I---KE---D---A---D---A---	: 291
TV008c4.4	: K---TE	: TV---S---E---N---I---I---E---D---A---D---A---	: 288
TV019c5	: N---RE	: TM---S---T---S---N---S---KE---K---D---A---	: 293
TV003cE260	: E---OK	: S---TI---T---E---E---A---I---	: 276
TV004cC300	: F---E	: M---M---S---KE---	: 285
TV013cH17	: R---E	: I---TI---S---F---ED---R---S---E---I---	: 296
TV013cB20	: R---E	: I---TI---S---F---ED---R---S---E---I---V---	: 296

FIGURE 100 (Sheet 3 of 9)



gp120 ← || → gp41

```

IN21068      : L Y K Y K V V E K P L G V A P T T A K R R V V E R E K R A V G I G A V F L G F L G A A G S T M G A A S I T L T V Q A R Q L L S G T V Q Q Q S N L L R A T E A Q Q H L L Q L T V W G I K Q L Q T R V L A I E R Y L : 594
96EW05.02   : . . . . . I . . . . . E . . . . . C . . . . . L . . . . . N . . . . . I . . . . . V . . . . . I . . . . . V . . . . . : 580
ETH2220     : . . . . . I . . . . . K P . . . . . A - L . L . . . . . V . . . . . M . . . . . H . . . . . : 575
92BR025.8   : . . . . . I . . . . . I . . . . . K . . . . . K . . . . . M . . . . . A . . . . . : 580

TV001c8.2   : . . . . . I . . . . . I . . . . . K . . . . . Q . . . . . Q . . . . . K . . . . . M . . . . . A . . . . . : 591
TV001c8.1   : . . . . . I . . . . . I . . . . . K . . . . . Q . K . . . . . K . . . . . M . . . . . A . . . . . : 593
TV002c12.1  : . . . . . I . . . . . I . . . . . A . . . . . A . . . . . I . . . . . V . . . . . M . . . . . A . . . . . : 578
TV012c2.1   : . . . . . I Q . . . . . K . . . . . K . . . . . A - L . . . . . A - L . . . . . V . . . . . M . . . . . : 569
TV012c2.2   : . . . . . I Q . . . . . K . . . . . K . . . . . A - L . . . . . A - L . . . . . T . . . . . M . . . . . : 575
TV006c9.1   : . . . . . I . . . . . I . . . . . I . . . . . N . . . . . T L . M . . . . . A . . . . . V . . . . . M . . . . . : 581
TV006cE9    : . . . . . I . . . . . I . . . . . I . . . . . N . . . . . T L . M . . . . . A . . . . . K . . . . . M . . . . . : 577
TV006c9.2   : . . . . . I . . . . . I . . . . . I . . . . . N . . . . . T L . M . . . . . A . . . . . K . . . . . V . . . . . : 583
TV007cB104  : . . . . . I . . . . . I . . . . . I . . . . . A . . . . . A . L . L . . . . . A . . . . . K . . . . . : 569
TV007cB105  : . . . . . I . . . . . I . . . . . I . . . . . A . . . . . A . L . L . . . . . A . . . . . K . . . . . : 583
TV010cD7    : . . . . . I . . . . . I . . . . . I . . . . . M . . . . . M . . . . . P F . . . . . M . . . . . : 589
TV018cF1027 : . . . . . I . . . . . I . . . . . E . . . . . E . . . . . G . . . . . L . . . . . L . . . . . M . . . . . : 589
TV014c6.3   : . . . . . I . . . . . I . . . . . I . . . . . E . . . . . E . . . . . L . . . . . L . . . . . Y M . . . . . : 589
TV014c6.4   : . . . . . I . . . . . I . . . . . I . . . . . A . . . . . A . . . . . I . . . . . I . . . . . M . . . . . : 586
TV008c4.3   : . . . . . I . . . . . I . . . . . I . . . . . A . . . . . A . . . . . I . . . . . I . . . . . M . . . . . : 593
TV008c4.4   : . . . . . I . . . . . I . . . . . I . . . . . G . . . . . G . . . . . L . M . . . . . L . M . . . . . : 569
TV019c5     : . . . . . I . . . . . I . . . . . I . . . . . N K . . . . . N K . . . . . A . . . . . V . . . . . : 579
TV003cE260  : . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . A . . . . . : 584
TV004cC300  : . . . . . I R . . . . . I . . . . . E . . . . . E . . . . . I . . . . . I . . . . . A . . . . . : 584
TV013cH17   : . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . A . . . . . : 584
TV013cB20   : . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . I . . . . . A . . . . . : 584

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FIGURE 100 (Sheet 6 of 9)


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IN21068      : LIGLRIIFAVISIVNRQGYSPLSFQTLTPNPGDPDLGRIEEGEGEQDKDRSIRIVSGFLALFWDDLENLCLFSYHRLRDFTLVAARVLELLGRSIRGLQRC : 803
96BW05.02    : .....L.....P.....RE.....RG.....A.....S.....I.....Q----- : 782
ETH2220      : .....L.....I.H.R.....G.....CR.....N.....I.....S.....L.....I.....TV.....S.K..... : 784
92BR025.8    : .....L.....R.....G.....R.....A.....S.....L.....I.....AV.....S.....I..... : 789

TV001c8.2    : .....L.....S.R.L.....G.....R.....S.A.....I.V.AV.....HS..... : 800
TV001c8.1    : .....L.....S.R.L.....G.....R.....S.A.....I.V.AV.....HS..... : 802
TV002c12.1   : .....L.....L.....I.....R.....G.....SS.....T.A.....S.....C.....IVV.AV.....HS..... : 787
TV012c2.1    : .....L.....L.....AQ.R.....T.....R.....R.....A.E.....I.L.....V.T.AV.....S..... : 778
TV012c2.2    : .....L.....L.....AQ.R.....T.....R.....R.....A.E.....I.L.....V.T.AV.....S..... : 778
TV006c9.1    : .....S.L.....A.....REL.....R.....Q.....A.....S.....I.KAA.....HN..... : 784
TV006c9.2    : .....L.....I.....REL.....R.....R.....A.....S.....I.AA.....HS..... : 790
TV006c9.2    : .....L.....I.....REL.....R.....R.....Q.....A.....S.....N.....I.AA.....HS..... : 786
TV007cB104   : .....L.....I.S.....R.....LS.....R.....S.A.....S.....L.IVV.AV.....S..... : 792
TV007cB105   : .....L.....I.S.....R.....LS.....R.....S.A.....S.....L.IVV.AV.....S..... : 779
TV010cD7     : .....L.....A.....I.D.R.....P.....R.....V.N.....V.....S.....Q.....L.IV.AV.V.....N.....T..... : 792
TV018cF1027  : .....L.....L.....I.....R.....RE.....FS.A.....I.T.V..... : 791
TV014c6.3    : .....L.....L.....R.....RE.....FS.A.....IVT.V..... : 791
TV014c6.4    : .....L.....L.....I.D.R.....R.....V.....V.....A.....S.....L.....GV.....V.....S.K..... : 798
TV008c4.3    : .....L.....L.....I.D.R.....R.....V.....V.....A.....C.....L.....L.....GV.....V.....S.K..... : 795
TV008c4.4    : .....L.....L.....L.....K.....L.....R.....RG.....R.....V.....N.....IA.....S.....Q.....IV.AV.I..... : 795
TV019c5      : .....L.....L.....V.....I.S.R.....S.....R.....V.....N.....Q.....I.T.AV.....S..... : 778
TV003cE260   : .....L.....L.....V.....S.....I.....RE.....RG.....R.....V.....N.....Q.....S.R.....Q.....IV.AV.....QS..... : 788
TV004cC300   : .....L.....L.....K.....I.....RE.....RG.....R.....V.....N.....Q.....S.R.....Q.....IV.AV.....QS..... : 793
TV013cH17    : .....L.....L.....K.....I.....RE.....RG.....R.....V.....N.....Q.....S.R.....Q.....IV.AV.....QS..... : 793
TV013cB20    : .....L.....L.....K.....I.....RE.....RG.....R.....V.....N.....Q.....S.R.....Q.....IV.AV.....QS..... : 793

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FIGURE 100 (Sheet 8 of 9)

gp41 ←

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IN21068 : WEALKYLGSLVQYWGLELKKSAINLLDRIAIAVAEGTDRILELVQRICRAIRNIPRRIRQGFEEALQ : 870
96BW05.02 : .....S...T.....I.FI.....I.FI..... : 849
ETH2220 : ..T.....NTT..V.G.....FI..I..W..FC.....L..... : 851
92BR025.8 : ..I....G.....S...S.F.T.....I.VI.G.W...C..... : 856

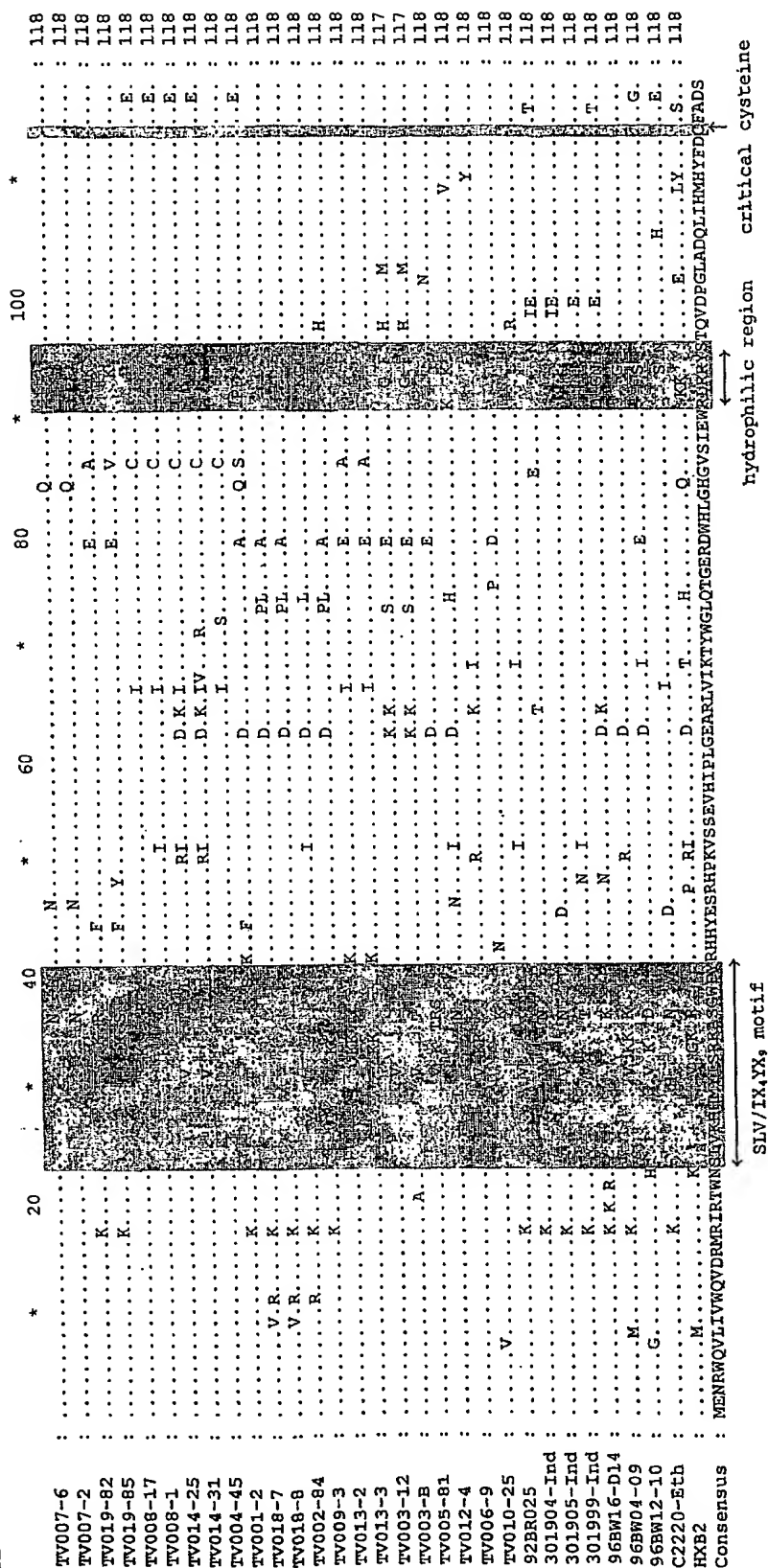
TV001c8.2 : ..I.....S...T...T.....I.....L.....L : 867
TV001c8.1 : ..I.....SP..T.....I.....L.....L : 869
TV002c12.1 : ..GT.....T.....FI.NL..G...V..... : 854
TV012c2.1 : ..I.....VS...SL.....I.FL.G.G...Y..... : 845
TV012c2.2 : ..I.....VS...SL.....I.FL.G.G...Y..... : 845
TV006c9.1 : ..I....A.....S...T.....I..I..W...T..... : 851
TV006cE9 : ..I....A.....S...IT.....I..I..W...T...L : 857
TV006c9.2 : ..I....A....R..S...IT.....I..I..W...T..... : 853
TV007cB104 : .....G..... : 803
TV007cB105 : .....G..... : 803
TV010cD7 : .....S...T...T.....I..I.G.G...Y..... : 846
TV018cF1027 : .....N..L.....R...S...TT..V.....F.AIC.....R.....L : 859
TV014c6.3 : ..T.....G.....S...A.....I.FI.....T...H... : 857
TV014c6.4 : ..T.....G.....R...S...A...V.....I.FI.....T..... : 858
TV008c4.3 : .....E...S...T...T.G...I.FL.....L...H..... : 865
TV008c4.4 : .....S...T...T.G...I.FL.....L..... : 862
TV019c5 : .....T.....I..ILGLG...C..... : 862
TV003cE260 : .....VS..TV..V.....I...V.....T...L : 845
TV004cC300 : .....TS...T...T.....I..I..F...LH.....L : 855
TV013cH17 : .....N.....S...T.....V.II.....L : 860
TV013cB20 : .....N.....S...T.....I..II.....L : 860

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FIGURE 100 (Sheet 9 of 9)

Figure 101

A



A (continued)

	120	*	140	*	160	*	180	*	
TV007-6	:	: 192
TV007-2	:	: 192
TV019-82	:	: 192
TV019-85	:	: 192
TV008-17	:	: 192
TV008-1	:	: 192
TV014-25	:	: 192
TV014-31	:	: 192
TV004-45	:	: 192
TV001-2	:	: 192
TV018-7	:	: 192
TV018-8	:	: 192
TV002-84	:	: 192
TV009-3	:	: 192
TV013-2	:	: 192
TV013-3	:	: 192
TV003-12	:	: 191
TV003-B	:	: 191
TV005-81	:	: 192
TV012-4	:	: 192
TV006-9	:	: 192
TV010-25	:	: 192
92BR025	:	: 192
301904-Ind	:	: 192
301905-Ind	:	: 192
301999-Ind	:	: 192
96BW16-D14	:	: 192
96BW04-09	:	: 192
96BW12-10	:	: 192
C2220-Eth	:	: 192
HXB2	:	: 192
Consensus	:	: 192

critical cysteine

SLQYLA motif

phosphorylation sites

B

	*	20	*	40	*	60	*	80	*	
TV007-6	:	: 96
TV007-2	:	: 96
TV019-82	:	: 96
TV019-85	:	: 96
TV008-17	:	: 96
TV008-1	:	: 96
TV014-25	:	: 96
TV014-31	:	: 96
TV004-45	:	: 96
TV001-2	:	: 96
TV018-7	:	: 96
TV018-8	:	: 96
TV002-84	:	: 96
TV009-3	:	: 96
TV013-2	:	: 96
TV013-3	:	: 96
TV003-12	:	: 96
TV003-B	:	: 96
TV005-81	:	: 96
TV012-4	:	: 96
TV006-9	:	: 96
TV010-25	:	: 96
92BR025	:	: 96
301904-Ind	:	: 96
301905-Ind	:	: 96
301999-Ind	:	: 96
96BW16-D14	:	: 96
96BW04-09	:	: 96
96BW12-10	:	: 96
C2220-Eth	:	: 96
HXB2	:	: 96
Consensus	:	: 96

alpha helix domain, for nuclear localisation and virion incorporation

Leu/Ile rich domain, leucine zipper-like domain - interaction with cellular proteins

/\HXB2 frameshift

Figure 101

C

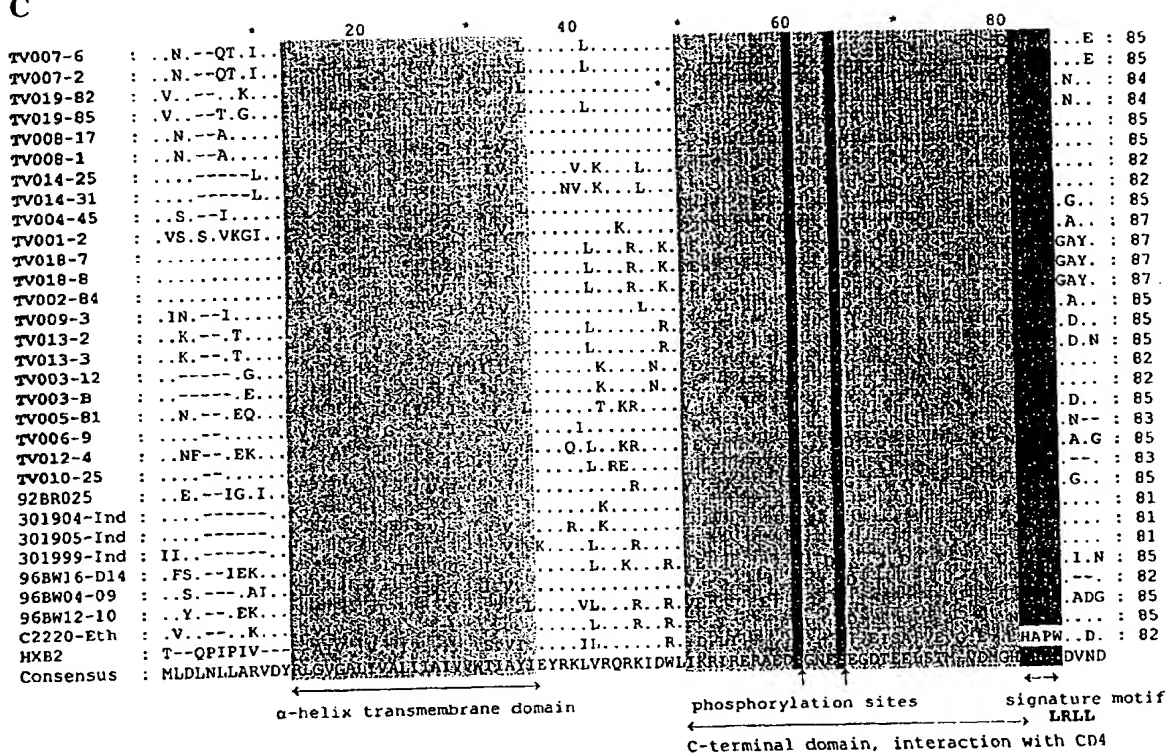


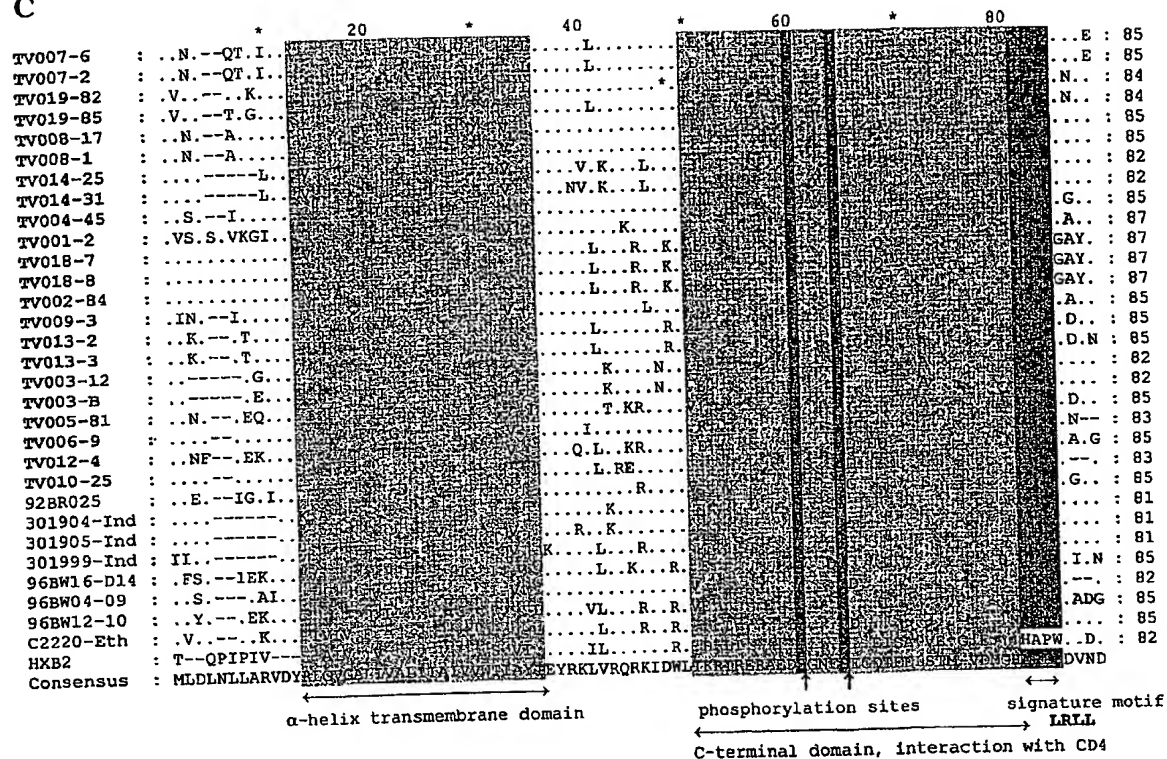
FIGURE 102 (SEQ ID NO:181)
Sheet 1 OF 2

3'half#8_2_TV1_C.ZA

GTCGACTGTAGTCCAGGAATATGGCAATTAGATTGTACACATTTAGAAGGAAAAATCATCCT
GGTAGCAGTCCATGTAGCTAGTGGCTACATAGAGGCAGAGGTTATCCCAGCAGAAACAGG
ACAAGAAACAGCATATTTTATATTTAAAATTAGCAGGAAGATGGCCAGTCAAGGTAATACATA
CAGACAATGGCAGTAATTTTACCAGTGCTGCAGTTAAGGCAGCCTGTTGGTGGGCAGGTAT
CCAACAGGAATTTGGAATTCCTTACAATCCCCAAAGTCAGGGAGTGGTAGAATCCATGAAT
AAAGAATTAAGAAAAATAATAGGACAAGTAAGAGATCAAGCTGAGCACCTTAGGACAGCAG
TACAAATGGCAGTATTCATTACAAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
AGGGGAAAGAATAATAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACAAA
TTATAAAAATTCAAAATTTTCGGGTTTATTACAGAGACAGCAGAGACCCTATTTGGAAAGGA
CCAGCCAAACTCTCTGAAAGGTGAAGGGGCAGTAGTAATAGAAGATAAAGGTGACATAA
AGGTAGTACCAAGGAGGAAAGCAAAAATCATTAGAGATTATGGAAAACAGATGGCAGGTGC
TGATTGTGTGGCAGGTGGACAGGATGAAGATTAGAGCATGGAATAGTTTAGTAAAGCACCA
TATGTATATATCAAGGAGAGCTAGTGGATGGTCTACAAACATCATTTTGAAGCAGACATC
CAAAAGTAAGTTCAGAAGTACATATCCCATTAGGGGATGCTAGATTAGTAATAAAAAACATAT
TGGGGTTTGCAGACAGGAGAAAGAGATTGGCATTGGGTCTGGAGTCTCCATAGAATGG
AGACTGAGAGAATATAGCACACAAGTAGACCCTGGCCTGGCAGACCAGCTAATTCATATGC
ATTATTTTGATTGTTTTACAGAATCTGCCATAAGACAAGCAATATTAGGACACATAGTTATCC
CTAGGTGTGACTATCAAGCAGGACATAAGAAGGTAGGATCTCTACAATACTTGGCACTGAC
AGCATTGATAAAACCAAAAAGGAGAAAGCCACCTCTGCCTAGTGTTAGGAAATTAGTAGAG
GATAGATGGAACGACCCCCAGAAGACCAGGGGCCGAGAGGGAACCATACAATGAATGG
ACACTAGAGATTCTAGAAGAACTCAAGCAGGAAGCTGTCAGACACTTTCCTAGACCATGGC
TCCATAACTTATGAAACCTATGGGGATACTTGGACGGGAGTTGAAGCTATAATAAGAGTAC
TGCAACAACACTACTGTTTCATTTCATTTCAGAATTGGATGCCAACATAGCAGAATAGGCATTTTG
CAACAGAGAAGAGCAAGAAATGGAGCCAGTAGATCCTAACTAGAGCCCTGGAACCATCC
AGGAAGCCAACCTAAAACTGCTTGTAAATTTGCTTTTGCAAACACTGTAGCTATCATTGTC
TAGTTTGCTTTCAGACAAAAGGCTTAGGCATTTTCCTATGGCAGGAAGAAGCGGAGACAGCG
ACGAAGCGCTCCTCCAAGTGGTGAAGATCATCAAAATCCTCTATCAAAGCAGTAAGTACTC
ATAGTAGATGTAATGGTAAGTTTAAGTTTAGATAAAGGAATAGATTATAGATTAGGAGTAGG
AGCATTAAATAGTAGCACTAATCATAGCAATAATAGTGTGGACCATAGTATATATAGAATATAA
GGAAATTGGTAAGACAAAAGAAAATAGACTGGTTAATTAAAAGAATTAGGGAAAGAGCAGA
AGACAGTGGCAATGAGAGTGATGGGGACACAGAAGAATTGTCAACAATGGTGGATATGGG
GCATCTTAGGCTTCTGGATGCTAATGATTTGTAACACGGAGGACTTGTGGGTCACAGTCTA
CTATGGGGTACCTGTGTGGAGAGACGCAAAAACACTCTATTCTGTGCATCAGATGCTAAA
GCATATGAGACAGAAGTGCATAATGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCA
ACCCACAAGAAATAGTTTTGGGAAATGTAACAGAAAATTTAATATGTGGAAAAATGACATG
GCAGATCAGATGCATGAGGATGTAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAA
AGTTGACCCCACTCTGTGTCACTTTAACTGTACAGATACAAATGTTACAGGTAATAGAAT
GTTACAGGTAATAGTACCAATAATACAAATGGTACAGGTATTTATAACATTGAAGAAATGAA
AAATTGCTCTTTCAATGCAACCACAGAATTAAGAGATAAGAAACATAAAGAGTATGCACTCT
TTTATAGACTTGATATAGTACCCTTAATGAGAATAGTGACAACCTTACATATAGATTAATAA
ATTGCAATACCTCAACCATAACACAAGCCTGTCCAAAGGTCTCTTTTGACCCGATTCTCTATA
CATTACTGTGCTCCAGCTGGTTATGCGATTCTAAAGTGAATAATAAGACATTCAATGGGAC
AGGACCATGTTATAATGTCAGCACAGTACAATGTACACATGGAATTAAGCCAGTGGTATCA
ACTCAATTACTGTTAAATGGTAGTCTAGCAGAAGAAGGGATAATAATTAGATCTGAAAATTT
GACAGAGAATACCAAAAACAATAATAGTACACCTTAATGAATCTGTAGAGATTAATTGTACAA
GACCCAACAATAATACAAGAAAAAGTGAAGGATAGGACCAGGACAAGCATTCTATGCAAC

Figure 101

C



AAATGATGTAATAGGAAACATAAGACAAGCACATTGTAACATTAGTACAGATAGATGGAACA
AAACTTTACAACAGGTAATGAAAAAATTAGGAGAGCATTTCCTAATAAAACAATACAATTTA
AACCACATGCAGGAGGGGATCTAGAAATTACAATGCATAGCTTTAATTGTAGAGGAGAATT
TTTCTATTGTAATACATCAAACCTGTTTAATAGCACATACCACTCTAATAATGGTACATACAA
ATACAATGGTAATTCAAGCTCACCCATCACACTCCAATGTAAAATAAAACAAATTGTACGCA
TGTGGCAAGGGGTAGGACAAGCAACGTATGCCCCTCCCATTGCAGGAAACATAACATGTA
GATCAAACATCACAGGAATACTATTGACACGTGATGGAGGATTTAACACCACAAACAACAC
AGAGACATTCAGACCTGGAGGAGGAGATATGAGGGATAACTGGAGAAGTGAATTATATAAA
TATAAAGTAGTAGAAATTAAGCCATTGGGAATAGCACCCACTAAGGCCAAAAAGAAGAGTGG
TGCAGAGAGAAAAAGAGCAGTGGGAATAGGAGCTGTGTTCTTGGGTTCTTGGGAGCAG
CAGGAAGCACTATGGGCGCAGCGTCAATAACGCTGACGGTACAGGCCAGACAACCTGTTGT
CTGGTATAGTGCAACAGCAAAGCAATTTGCTGAAGGCTATAGAGGCGCAACAGCATATGTT
GCAACTCACAGTCTGGGGCATTAAAGCAGCTCCAGGCGAGAGTCTTGGCTATAGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGCTGCTCTGGAAGACTCATCTGCACCACT
GCTGTGCCCTTGAACTCCAGTTGGAGTAATAAATCTGAAAAAGATATTTGGGATAACATGA
CTTGGATGCAGTGGGATAGAGAAATTAGTAATTACACAGGCTTAATATACAATTTGCTTGAA
GACTCGCAAACCAGCAGGAAAAGAATGAAAAAGATTTATTAGAATTGGACAAGTGGAAACA
ATCTGTGGAATTGGTTTGACATATCAAACCTGGCCGTGGTATATAAAAATATTCATAATGATA
GTAGGAGGCTTGATAGGTTTAAGAATAATTTTTGCTGTGCTTTCTATAGTGAATAGAGTTAG
GCAGGGATACTCACCTTTGTCATTTGACACCCTTACCCCAAGCCCGAGGGGACTCGACAG
GCTCGGAGGAATCGAAGAAGAAGGTGGAGAGCAAGACAGAGACAGATCCATACGATTGGT
GAGCGGATTCTTGTCGCTTGCCTGGGACGATCTGCGGAACCTGTGCCTCTTCAGCTACCA
CCGCTTGAGAGACTTCATATTAATTGCAGTGAGGGCAGTGGAACTTCTGGGACACAGCAGT
CTCAGGGGACTACAGAGGGGGTGGGAAATCCTTAAGTATCTGGGAAGTCTTGTGCAATATT
GGGGTCTAGAGCTAAAAAGAGTGCTATTAGTCTGCTTGATACCATAGCAATAACAGTAGC
TGAAGGAACAGATAGGATTATAGAATTAGTACAAAGAATTTGTAGAGCTATCCTCAACATAC
CTAGAAGAATAAGACAGGGCTTTGAAGCAGCTTTGCTATAAAATGGGGGGCAAGTGGTCAA
AATGCAGCGGATGGCCTGCAGTAAGAGAAAGAATGAGACGAGCTGAGCCAGCAGCAGAG
GGAGTAGGACCAGCGTCTCAAGACTTAGATAGACATGGGGCACTTACAAGCAGCAACACA
CCTGCCAATAATGATGCTTGTGCCTGGCTGCAAGCACAGGAGGACGGAGATGTAGGC
TTTCCAGTCAGACCTCAGGTACCTTTAAGACCAATGACTTATAAGAGCGCATTCGATCTCAG
CTTCTTTTTAAAGAAAAGGGGGGACTGGATGGGTTAGTTTACTCTAAGAAAAGGCAAGAA
ATCCTTGATTTGTGGGTCTATAACACACAAGGCTTCTCCCTGATTGGCAAACTACACACC
GGGGCCAGGGGTGAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTGCCAGTTGA
CCCAGGGGAGGTGGAAGAGGCCAACGGAGGAGAAGACAACCTGTTTGCTACACCCTATGA
GCCAACATGGAGCAGAGGATGAAGATAGAGAAGTATTAAAGTGGAAGTTTGACAGTCTCCT
AGCACGCAGACACATGGCCCCGCGAGCTACATCCGGAGTATTACAAAGACTGCTGACACAG
AAGGGACTTTCCGCCTGGGACTTTCCACTGGGGCGTTCCGGGAGGTGTGGTCTGGGCGG
GACTTGGGAGTGGTCAACCCTCAGATGCTGCATATAAGCAGCTGCTTTTCGCTTGACTGG
GTCTCTCTCGGTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTATCTAGGGAACCCACT
GCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTTAAGTAGTGTGTGCCCGTCTGTTGTGT
GACTCTGGTAAGTAGAGATCCCTCAGACCCTTTGTGGTAGTGTGGAAAATCTCTAGCAGCG
GCCGC

FIGURE 102 (SEQ ID NO:181)
Sheet 2 OF 2

FIGURE 103 (SEQ ID NO:182)
(Sheet 1 of 5)

Full#2_1/4_TV12_C_ZA

TGGAAGGGTTAATTTACTCTAATAAAAGGCAAGAGATCCTTGATTTGTGG
GTTTATAACACACAAGGCTTCTTCCCTGATTGGCAAACTACACACCGGG
GCCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGAGC
CAGTCGATCCAAAGGAAGTAGAAGAGGCCAATGAAGGAGAAAACAAGT
TTACTACACCCTATGAGCCAGCATGGGATGGAGGATGAAGACAGAGAAG
TATTAAGATGGAAGTTTGACAGTATGCTAGCACGCAGACACATGGCCCGC
GAGCTACATCCGGAGTATTACAAGGACTGCTGACACAGAAGGGACTTTC
GCTGGGACTTTCCACTGGGGCGTTCCAGGAGGTGTGGTCTGGGCGGGACT
GGGGAGTGGTCAGCCCTGAGATGCTGCATATAAGCAGCTGCTTTTCGCCT
GTACTGGGTCTCTCTAGGTAGACCAGATCTGAGCCCGGGAGCTCTCTGGCT
ATCTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCCTT
GAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCA
GACCACTTGTGGTGTGTGGAAAATCTCTAGCAGTGGCGCCTGAACAGGGA
CTTGAAAGCGAAAAGTAAGACCAGAGGAGATCTCTCGACGCAGGACTCGG
CTTGCTGAAGTGCACCTCGGCAAGAGGCGAGAGAGGCGGCTGGTGAGTAC
GCCAAATTTTATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGA
GAGCGTCAGTATTGAAAGGGAAAAAATTAGATACATGGGAAAGAATTAG
GTTAAGGCCAGGGGGAAAGAAACACTATATGCTAAACACCTAGTATGG
GCAAGCAGGGAGCTGGAAAGATTTGCACTTAACCCTGGCCTTTTAAAGAAC
AGCAGAAGGCTGTAAACAAATAATGCAACAGCTACAATCAGCTCTTCAGA
CAGGAACAGAGGAACCTTAGATCATTATATAACACAGTAGCAACTCTCTAT
TGTGTACATAAAGAGATAGATGTACGAGACACCAAGGAAGCCTTAGACA
AGATAGAGGAAGAACAATAAAGAGTCAGCAAAAAACACAGCAAGCAG
AAGCGGCTGACAAAGGAAAGGTCAGTCAAAATTATCCAATAGTGCAGAA
TCTCCAAGGGCAAATGGTACACCAGGCCATATCACCGAGAACTTTAAATG
CATGGGTAAAAGTAATAGAAGAGAAGGCTTTCAGCCCAGAGGTAATACCC
ATGTTTACAGCATTATCAGAAGGAGCTACCCCAAGATTTAAACACCAT
GTTAAATACAGTGGGGGGACACCAAGCAGCCATGCAAATGTTAAAGAT
ACCATCAATGAGGAGGCTGCAGAATGGGATAGGTTACATCCAGTGCATGC
AGGGCCTATTGCACCAGGCCAAATGAGAGAACCAAGGGGAAGTGACATA
GCAGGAACCTACTAGTACCCTTCAAGAACAAATAGCATGGATGACAAGTAA
CCCACCTATTCCGGTGGGAGACATCTATAAAAGATGGATAATTCTGGGGT
TAAATAAAATAGTAAGAATGTATAGCCCTGTCAGCATTTTGGACATAAAA
CAAGGGCCAAAAGAACCCTTTAGAGACTATGTAGACCGATTCTTTAAAC
TTTAAGGGCTGAACAATCTTCACAAGAGGTAAAAAATTGGATGACAGACA
CCTTGTGGTCCAAAATGCAAACCCAGATTGTAAGACCATTTTAAGAGCA
TTAGGACCAGGGGCTACATTAGAGGAAATGATGACAGCATGTCAGGGAGT
AGGAGGACCTGGCCACAAAGCAAGAGTTTGGCTGAGGCAATGAGCCAA
GCAAATACAAACATAATGATGCAGAAAAGCAATTTTAAAGGCCCTAAAA
GAACTGTTAAATGTTTCAATTGTGGCAAGGAAGGGCATATAGCCAGAAAT
TGCAGGGCCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGAC
ACCAATGAAAGACTGTACTGAAAGGCAGGCTAATTTTTTAGGGAAAATT
TGGCCTTCCTACAAGGGGAGGCGGGGAATTTCTTCAGAGCAGACCAGA
ACCATCAGCCCCACCAGCAGAGAGCTTCAGGTTTCGAGGAGCAGGAGCCG
AAAGACAAGGAACCAACCCTTAACCTCCCTCAAATCACTCTTTGGCAGCGA
CCCCTTGTCTCAATAAAAGTAGAGGGCCAGATAAAGGAGGCTCTCTTAGA
TACAGGAGCAGATGATACAGTATTAGAAGAAATAAATTTGCCAGGAAAT

FIGURE 103 (SEQ ID NO:182)

(Sheet 2 of 5)

GGAAACCAAAAATGATAGGAGGAATTGGAGGTTTTATCAAAGTAAGACA
GTATGAGCAAATACTTATAGAAATTTGTGGAAAAAAGGCTATAGGAACAG
TATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATATGTTGACT
CAGCTTGGATGCACACTAAATTTTCCAATTAGTCCCATTGAACTGTACCA
GTAAAATTAAGCCAGGAATGGATGGCCCAAGAGTTAAACAATGGCCATT
GACAGAAGAAAAAATAAAAGCATTAAACAGCAATTTGTGAAGAAATGGAG
AAGGAAGGAAAAATTACAAAAATTGGGCCTGAAAATCCATATAACACTCC
AGTATTTGCCATAAAAAAGAAGGACAGTACTAAGTGGAGAAAATTAGTA
GATTTTCAGGGAAGTCAATAAAAGAACTCAAGACTTTTGGGAAGTTCATT
AGGAATACCACACCCAGCAGGGTTAAAAAAGAAAAAATCAGTGACAGTG
CTGGATGTGGGGGATGCATATTTTTCAGTTCCTTTAGATGAAAGCTTCAGG
AAATATACTGCATTACCATACCTAGTATAAACAATGAAGCACCAGGGAT
TAGATATCAATATAATGTGCTTCCACAGGGGTGGAAAGGATCACCAGCAA
TATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTATAGGAAACAAAAT
CCAAACATAGTTATCTATCAATATATGGATGATTTGTATGTAGGATCTGAC
TTAGAAATAGGGCAACATAGAGCAAAAATAGAGGAGTTAAGAGAACATT
TATTGAGGTGGGGACTTACCACACCAGACAAGAAACATCAGAAAGAACC
CCCATTTCTCTGGATGGGGTATGAACTACATCCTGACAAATGGACAGTAC
AGCCTATACTGCTGCCAGAAAAGGATAGCTGGACTGTCAATGATATACAG
AAGTTAGTGGGAAAGTTAACTGGGCCAGTCAGATTTACCCAGGGATTAA
AGTAAAGTACTTGTGCAAACTCCTTAGGGGAGCCAAAGCACTAACAGACA
TAGTACCACTGACTGAAGAAGCTGAATTAGAATTGGCAGAGAACAGGGA
AATTCTAAAAGAACCAGTACATGGAGTATATTATGACCCCTCAAAAGACT
TAATAGCTGAAATACAGAAACAGGGGCATGACCAATGGACATACCAAATT
TACCAAGAACCATTCAAAAATCTGAAAACAGGGAAGTATGCAAAAATGA
GGACTGCCCACACTAATGATGTAAAACAGTTAACAGAAGCAGTGCAAAA
AATAGCTCTAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTCAGAC
TACCCATCCAAAAAGAAACATGGGAGACATGGTGGACAGACTATTGGCA
AGCCACCTGGATCCCTGAATGGGAGTTTGTTAATACCCCTCCCCTAGTAAA
ATTATGGTACCAACTGGAAAAAGAACCCATAGCAGGGGTAGAGACTTTCT
ATGTAGATGGAGCAGCTAACAGGGAACTAAAATAGGAAAAGCAGGGTA
TGTTACTGACAAAGGAAGACAGAAAATTGTACTCTAAATGAAACAACAA
ATCAGAAGGCTGAGTTACAAGCAATTCAGCTAGCTTTGCAGGATTCAGGA
TCAGAAGCAAACATAGTAACAGACTCACAGTATGCATTAGGAATTATTCA
AGCACAACCAGATAAGAGTGAATCAGAGTTAGTTAACCAGATAATAGAA
CAGTTAATAAACAAGGAGAGAATCTACCTGTCATGGGTACCAGCACATAA
AGGAATTGGAGGAAATGAACAAGTAGACAAATTAGTAAGTAGTGGAATC
AGGAAAGTGCTGTTTCTAGATGGGATAGATAAGGCTCAAGAAGAGCATGA
AAAATATCACAGCAATTGGAGAGCAATGGCTAGTGAGTTAATCTGCCAC
CCATAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAA
GGGAAGCCATACATGGACAAGTCGACTGTAGTCCAGGAATATGGCAATT
AGATTGTACACATTTAGAAGGAAAAATCATCCTGGTAGCAGTCCATGTAG
CCAGTGGCTACATAGAAGCAGAGGTTATCCCAGCAGAAACAGGACAAGA
AACAGCATATTATATACTAAAATTAGCAGGAAGATGGCCAGTTAAAATAA
TACATACAGATAATGGCAGTAATTTACCAGTGCTGCAGTTAAAGCAGCC
TGTTGGTGGGCAGGAATCCAACAGGAATTTGGAATTCCTACAATCCCCA
AAGTCAGGGAGTAGTAGAATCCATGAATAAAGAATTAAAGAAAATCATA
GGGCAGGTAAGAGATCAAGCTGAGCACCTCAAGACAGCAGTACAAATGG

FIGURE 103 (SEQ ID NO:182)

(Sheet 3 of 5)

CAGTATTCATTACACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGT
GCAGGGGAAAGGATAATAGACATAATAGCAACAGACATACAACTAGAG
AATTACAAAAACAAATTATAAAAATTCAAAATTTTCGGGTTTATTACAGG
GACAGCAGAGACCCTATTTGGAAGGACCAGCCAACTACTCTGGAAAG
GTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAGGTAGTACC
AAGGAGGAAAGTAAAAATCATTAAAGGACTATGGAAAACAGATGGCAGGT
GCTGATTGTGTGGCAGGTAGACAGGATGAAGATTAGAACATGGAATAGTT
TGGTAAAGCATCACATATATATTTCAAGGAGAGCTAATGGATGGTTTTAC
AGACATCATTATGAAAGCAGACACCCAAAAATAAGTTCAGAAGTACACAT
CCCATTAGGGGATGCTAGATTAGTAATAAAAACATATTGGGGTTTGCATA
CAGGAGAAAAGAGATTGGCATTGTTGGGTCATGGAGTCTCCATAGAATGGAAA
TTGAGAAAATATAGCACACAAGTAGACCCTGGCCTGGCAGACCAGCTAAT
TCATGTGCATTATTTTGATTGTTTTGCAGACTCTGCCATAAGACAAGCCAT
ATTAGGACACATAGTTATTCCTAGGTGTGACTATCAAGCAGGACATAATA
AGGTAGGATCTCTACAATACTTGGCACTGACAGCATTGATAAAACCAAAA
AAGAGAAAGCCACCTTTGCATAGTGTAGGAAATTAGTAGAGGATAGATG
GAACAAGCCCCAGAAGACCAGGGACCGCAGAGGGAACCATACAATGAAT
GGACACTAGAGCTTTTAGAGGAACTCAAACAGGAAGCTGTCAGACACTTT
CCTAGACCATGGCTCCATAGCTTAGGGCAACATATCTATAACACCTATGG
GGATACTTGGACAGGAGTAGAAGCTATAATAAGAATTCTGCAACAACCTAC
TGTTTATTTCATTTTCAAGAAATTGGGTGCCAGCATAGCAGAATAGGCATTATGC
GACAGAGAAGAGCAAGAAATGGAACCAGTAGATCCTAAACTTGAGCCCT
GGAAACATCCAGGAAGTCAGCCTAAACTCCTTGTAAATAATTGCTATTGC
AAAAAATGTAGCTATCATTGTCTAGTTTGCTTTTCAAGAAAAAGGCTTAGG
CATTTTCATATGGCAGGAAGAAGCGGAGACAACGACGAAGCACTCCTCCAA
GCAGTGAGGATCATCAAAATCTTATATCAAAGCAGTAAGTACTAAATGGT
AGATGTAATGTAAAGTTTTCTAGAAAAAGTAGATTATGAAATAGGAGTAG
CAGCATTTATAATAGCACTAATCATAGCAATAGTTGTGTGGATCATAGTAT
ATATAGAATATAGGAAATTGTTAAGACAAAAAGAATAGACTGGTTAATT
GAAAGAATTAGAGAAAGGGCAGAAGACAGTGGCAATGAGAGTGATGGGG
AGCAGGAGGAATTATCAACAATGGTGGATATGGGGAATCTTAGGCTTTTG
GATGCTAATGGTTGGTAATGTAATGGGGAACCTGTGGGTCACAGTCTATT
ATGGGGTACCTGTGTGGAAAGACGCAAAAGCTACTCTATTTTGTGCATCT
GATGCTAAAGCATATGAGAAAGAAGTGCATAATGTCTGGGCTACACATGC
CTGTGTACCCACAGACCCCGACCCACAAGAAATAGTTTTGGAGAATGTAA
CAGAAAATTTTAACATGTGGAATAAACATGGTGGACCAGATGCATGAG
GATATAATCAGCTTATGGGATCAAAGCCTAAAGCCATGTGTAAAGTTGAC
CCCACTCTGTGTCACTTTAACTGTAGCAATAATGTTAAAAATGCTACCAA
CAGTATGAAGGAAATGAAAAATTGCACCTTTCAATATAACCACAGAACTAA
GAGATAAGAGAAAGCAAGAATATGCACCTTTTTATAAACTTGATATAGTA
CCACTTGAGGAGAATTCCAGTAAGTATAGATTAATAAATTGTAATACCTC
AGCCATAACCCAAGCCTGTCCAAAGGTCTCTTTTGACCCAATTCTATACA
TTATTGTGCTCCAGCTGGTTATGCGATTCTAAAGTGTAATAATAAGACATT
CAATGGAACAGGACCATGCAATAATGTGACGACGGTACAATGTACACATG
GAATTAAGCCAGTAGTATCAACTCAACTACTGTAAATGGTAGTCTAGCA
GAAGAAGAAATAGTAATTAGATCTGAAAATATGACAAACAATGCCAAAA
TAATAATAGTACATCTTAATGAATCTGTAGAAATTACGTGTACAAGGCCC
AACATAATACAAGAAAAAGTATGAGGATAGGACCAGGACAAACATTCT

FIGURE 103 (SEQ ID NO:182)

(Sheet 4 of 5)

ATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACACTGTAACAT
TAGTGAAAAGCAATGGGATCAGACTTTATACAGGGTAAGTGAAAAATTAA
AAGAACACTTCCCTAATAAAACAATAAAGTTTAACTCATCCTCAGGAGGG
GACTTAGAAATTACAACACATAGCTTTAATTGTGGAGGAGAGTTTTTCTAT
TGCAATACATCTGTACTGTTTAATGGCACATACAGTAATGGCACAAACAG
TACAAATACAACAGTCATCACACTCCCATGCAGAATAAAACAAATTATAA
ACATGTGGCAGGGGGTAGGACGAGCAATGTATGCCCCTCCCATTGCAGGA
AACATAACATGTAGATCAAACATCACAGGACTAATATTGACACGTGATGG
AGGGCAGGGAGAGAATGACACAAATGAGATATTTAGACCTGCAGGAGGA
GATATGAGGGACAATTGGAGAAGTGAATTATACAAATATAAAGTGGTAG
AAATTGAGCCATTAGGAGTAGCACCCACTAAGGCCAAAAGGAGAGTGGT
GGAGAGAGAAAAAAGAGCAGCTTTGGGAGCTGTGTTCCCTTGGGTTCTTGG
GAGCAGCAGGAAGCACTATGGGCGCGGCATCAATAATGCTGACGGTACA
GGCCAGACAACTGTTGTCTGGTATAGTGCAACAGCAAAGCAATTTGCTGA
GAGCTGTAGAGGCGCAACAGCATATGTTGCAACTCACGGTCTGGGGCATT
AAGTAGCTCCAGACAAGAGTCCTGGCTATAGAAAGATACCTAAAGGATCA
ACAGCTCCTAGGGATTTGGGGCTGCTCTGGAAACTCATCTGCACCACTG
CCGTGCCTTGGAACAATAGTTGGAGTAATAAATCTCAAGATTATATTTGG
GGAAACATGACCTGGATGCAATGGGATAAAGAAATTAGCAATTACACAG
AAACAATATACAGGTTGCTTGGGGACGCGCAAAACCAGCAGGAGAAAAA
TGAAAAGGAGTTACTAGAATTGGACAGGTGGGGAAATCTGTGGAAGTGGT
TTGACATAACAAAATGGCTGTGGTATATAAAAATATTCATAATGGTAATA
GGAGGCTTGATAGGTTTAAGAATAATTTTTGCTGTGCTTTCTATAGTAAAT
AGAGTTAGGCAGGGATACTCACCTTTGTCAATTCAGACCCTTGCCCCAAAC
CCGAGGGGACCCGACAGGCTCGGAAGAACCGAAGAAGAAGGTGGAGAGC
AAGACAGAGACAGATCCATAAGATTAGTGAGCGGATTCTTAGCACTTGCC
TGGGAGGACCTGAGGAACCTGTGCATTTTCCTCTACCACCGATTGAGAGA
CTTCATATTGGTGACAGCGAGAGCAGTGGAACCTTCTGGGACGCAGCAGTC
TCAGGGGACTCCAGAGGGGGTGGGAAATCCTTAAGTACCTGGGAAGTCTT
GTGCAGTATTGGGGTCTAGAGCTAAAAAAGAGTGCTGTTAGTCTGCTTGA
TAGCGTAGCAATAGCAGTAGCTGAGGGAACAGATAGAATTATAGAATTCT
TACAAGGAACTGGTAGAGCTATCTACAACATACCTAGAAGAATAAGACAG
GGCTTTGAAGCAGCTTTGCAGTAAAATGGGAAATAAGTGGTCAAAAAGCT
GGCCTGCTGTAAGAGAAAGAATATGGAAACTAGGCCAGCAGCAGCAGA
AGCAGCTAGGCCAGCAGCAGCAGAAGGAGTAGGAGCAGCGTCTCAAGAC
TTGGATAAACGTGGGGCGCTTACAATCAACAACACAGCCAACAATAATCC
TGATTGTGCCTGGCTGGAAGCGCAAGAGGATGAGGAAGTAGGCTTTCCAG
TCAGACCTCAGGTACCTTTAAGACCAATGACATATAAGGCAGCATTTGAT
CTCAGCTTCTTTTTAAAGAAAAGGGGGGACTGGAAGGGTTAATTTACTC
CAGGAAAAGGCAAGAGATCCTTGATTTATGGGTCTATCACACACAAGGCT
ACTTCCCTGATTGGCAAACTACACACCGGGACCAGGGGTGAGATATCCA
CTGACCTTTGGATGGTGCTTCAAGCTAGTGCCAGTTGACCCAAGGGAAGT
AGAAGAGGCCAACGGAGGAGAAGACAACCTGTTTGCTACACCCTATGAGC
CAGTATGGAATGGATGATGAACACAAAGAAGTGCTACAGTGGAAGTTTGA
CAGCAGCCTAGCACGCAGACACCTGGCCCGCGAGCTACATCCGGATTATT
ACAAAGACTGCTGACACAGAAGGGACTTTCCGCCTGGGACTTTCCACTGG
GGCGTTCCAGGGGGAGTGGTCTGGGCGGGACTGGGAGTGGCCAGCCCTCA
GATGCTGCATATAAGCAGCTGCTTTTCGCCTGTACTGGGTCTCTCTAGGTA

FIGURE 103 (SEQ ID NO:182)

(Sheet 5 of 5)

GACCAGATCTGAGCCTGGGAGCTCTGTCTATCTGGGGAACCCACTGCTT
AAGCCTCAATAAAGCTTGCCTTGAGTGCTCTAAGTAGTGTGTGCCCATCTG
TTGTGTGACTCTGGTAACTCTGGTAACTAGAGATCCCTCAGACCCTTTGTG
GTAGTGTGGAAAATCTCTAGCA

FIGURE 104 (SEQ ID NO:183)

gp140.modTV1.mut1.dV2

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
 61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
 121 cccgtgtggc ggcacgcaa gaccacctg ttctgcgcca ggcacgcaa ggcctacgag
 181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
 241 gagatcgtgc tgggcaactg gaccgagaac ttaacatgt ggaagaacga catggccgac
 301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
 421 accggcaaca gcaccaaaaa caccaacggc accggcatct acaacatcga ggagatgaag
 481 aactgcagct tcaacgccgg cgcggccgc ctgatcaact gcaacaccag caccatcacc
 541 caggcctgcc ccaaggtgag ctgcacccc atcccatcc actactgcgc ccccgccggc
 601 tacgccatcc tgaagtga caacaagacc ttaacggca ccggccctg ctacaactg
 661 agcaccgtgc agtgaccca cggcatcaag ccgtgtgtga gacccagct gctgctgaac
 721 ggagcctgg ccgaggagg catcatcatc cgcagcgaga acctgaccga gaacaccaag
 781 accatcatc tgacctgaa cgagagcgtg gagatcaact gcaccgccc caacaacaac
 841 acccgcaaga gcgtgcgcat cggccccggc caggcctct acgccacca cgactgac
 901 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gacctgac
 961 cagtgatga agaagctggg cgagcactt cccaacaaga ccatccagtt caagccccac
 1021 gccggcggcg acctggagat caccatgcac agcttcaact gccgcggcga gtcttctac
 1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
 1141 aacggcaaca gcagcagccc catcaccctg cagtgaaga tcaagcagat cgtgcgcatg
 1201 tggcagggcg tgggccaggc cacctacgcc ccccccacg ccggcaacat cacctggcg
 1261 agcaacatca ccggcatcct gctgacccgc gacggcggct tcaacaccac caacaacac
 1321 gagaccttc gcccggcgg cgcgacatg cgcgacaact ggcgagcga gctgtacaag
 1381 tacaaggtgg tggagatcaa gccctgggc atgccccca ccaaggccaa gcgccgctg
 1441 gtgcagcgc agaagagcgc cgtgggcac gcgcccgtgt tctgggctt cctggcgcc
 1501 gccggcagca ccatgggcgc cgcagcatc acctgaccg tgcaggccc ccagctgctg
 1561 agcggcatc tgacgagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
 1621 ctgcagctga ccgtgtggg catcaagcag ctgaggccc gcgtgctggc catcgagcgc
 1681 tacctgaagg accagcagct gctgggcac tggggctgca gcggccgct gatctgacc
 1741 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
 1801 atgacctgga tcagtgagg ccgcgagatc agcaactaca ccggcctgat ctacaacctg
 1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
 1921 tggacaacc tgtggaactg gtcgacatc agcaactggc cctggtacat ctaa

FIGURE 105 (SEQ ID NO:184)

gp 140mod.TV1.mut2.dV2

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
 61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
 121 cccgtgtggc gcgacgcca gaccaccctg ttctgcgcca gcgacgcca ggcctacgag
 181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccg
 241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
 301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 acccccctgt gctgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
 421 accggcaaca gcaccaaca caccaacggc accggcatct acaacatcga ggagatgaag
 481 aactgcagct tcaacgccg cgccggccgc ctgatcaact gcaacaccag caccatcacc
 541 caggcctgcc ccaaggtgag ctgcacccc atcccatcc actactgccc ccccgccggc
 601 tacgccatcc tgaagtgcaa caacaagacc ttcaacggca ccggcccctg ctacaacgtg
 661 agcaccgtgc agtgaccca cggcatcaag cccgtggtga gcaccagct gctgctgaac
 721 ggagccttg ccgaggagg catcatcacc cgcagcgaga acctgaccga gaacaccaag
 781 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcacccgccc caacaacaac
 841 acccgcaaga gcgtgcgcat cgccccggc caggccttct acgccaccaa cgacgtgatc
 901 ggcaacatcc gccaggcca ctgcaacatc agcaccgacc gctggaacaa gaccctgcag
 961 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
 1021 gccggcggcg acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
 1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
 1141 aacggcaaca gcagcagccc catcacctg cagtgaaga tcaagcagat cgtgcgcatg
 1201 tggcagggcg tgggccaggc cacctacgcc cccccatcg ccggcaacat cacctgccg
 1261 agcaacatca ccggcatcct gctgacccgc gacggcggct tcaacaccac caacaacacc
 1321 gagaccttc gcccgggcgg cggcgacatg cgcgacaact ggcgagcga gctgtacaag
 1381 tacaaggtgg ttgagatcaa gccctgggc atgccccca ccaaggccaa gcgcccgtg
 1441 gtgcagagcg agaagagcgc cgtgggcac ggccgctgt tctgggctt cctgggcgc
 1501 gccggcagca ccatgggcgc cgccagcatc acctgaccg tgcaggcccg ccagctgctg
 1561 agcggcatcg tgcagcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
 1621 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
 1681 tacctgaagg accagcagct gctgggcac tggggctgca gcggccgct gatctgcacc
 1741 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
 1801 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
 1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
 1921 tggacaacc tgtggaactg gttcgacatc agcaactggc cctgttacat ctaa

FIGURE 106 (SEQ ID NO:185)

gp140mod.TV1.mut3.dV2

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
 61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgacctgtga ctacggcgtg
 121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
 181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
 241 gagatcgtgc tgggcaacgt gaccgagaac ttaacatgt ggaagaacga catggccgac
 301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 acccccctgt gcgtgaccct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
 421 accggcaaca gcaccaacaa caccaacggc accggcatct acaacatcga ggagatgaag
 481 aactgcagct tcaacgccgg cgccggccgc ctgatcaact gcaacaccag caccatcacc
 541 caggcctgcc ccaagtgtag ctgcacccc atccccatcc actactgccc ccccgccggc
 601 tacgccatcc tgaagtgcga caacaagacc ttcaacggca ccggccctg ctacaactg
 661 agcaccgtgc agtgacacca cggcatcaag ccctgtgtga gcaccagct gctgtgaac
 721 ggcagcctgg ccgaggaggg catcatcatc gcgagcgaga acctgaccga gaacaccaac
 781 accatcatcg tgacctgaa cgagagcgtg gagatcaact gacccgccc caacaacaac
 841 acccgcaaga gcgtgcgcat cggccccggc caggccttct acgccacaa cgacgtgatc
 901 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gacctgacg
 961 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
 1021 gccggcgggc acctggagat caccatgcac agcttcaact gccgcggcga gttcttctac
 1081 tgcaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
 1141 aacggcaaca gcagcagccc catcaccctg cagtgcgaaga tcaagcagat cgtgcgcatg
 1201 tggcagggcg tgggccaggc cacctacgcc cccccatcg ccggcaacat cacctgccgc
 1261 agcaacatca ccggcatcct gctgacccgc gacggcggct tcaacaccac caacaacacc
 1321 gagaccttc gccccggcgg cggcgacatg cgcgacaact ggcgcagcga gctgtacaag
 1381 tacaagggtg tggagatcaa gcccctgggc atgccccca ccaaggccaa gcgcagcgtg
 1441 gtgcagagcg agaagagcgc cgtgggcac gcgcgccgtgt tctgggctt cctgggcgcc
 1501 gccggcagca ccatgggcgc gccagcatc accctgaccg tgaggcccg ccagctgctg
 1561 agcggcatcg tgagcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
 1621 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcgagcgc
 1681 tacctgaagg accagcagct gctgggcac tggggctgca gcggccgcct gatctgcacc
 1741 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
 1801 atgacctgga tgagtgagg ccgcgagatc agcaactaca ccggcctgat ctacaacctg
 1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
 1921 tggacaacc tgtggaactg gttcgacatc agcaactggc cctgtacat ctaa

FIGURE 107 (SEQ ID NO:186)

gp140mod.TV1.mut4.dV2

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
 61 ttctgggatgc tgatgatctg caacaccgag gacctgtggg tgacctgta ctacggcgtg
 121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
 181 accgaggtgc acaacgtgtg ggccaccac ccctgcgtgc ccaccgacc caacccccag
 241 gagatcgtgc tgggcaactg gaccgagaac ttcaacatgt ggaagaacga catggccgac
 301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 acccccctgt gcgtgacct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
 421 accggcaaca gcaccaaaa caccaacggc accggcatct acaacatga ggagatgaag
 481 aactgcagct tcaacgccc gcggggccgc ctgatcaact gcaacaccag caccatcacc
 541 caggcctgcc ccaaggtgag ctgcgacccc atccccatcc actactgcgc ccccgccggc
 601 tacgccatcc tgaagtga caacaagacc ttcaacggca ccggcccctg ctacaacgtg
 661 agcaccgtgc agtgaccca cggcatcaag cccgtggtga gacccagct gctgctgaac
 721 ggcagcctgg ccgaggaggg catcatcacc cgcagcgaga acctgaccga gaacaccaag
 781 accatcatcg tgcacctgaa cgagagcgtg gagatcaact gcacccgccc caacaacaac
 841 acccgcaaga gcgtgcgcat cggcccccgc caggcctct acgccacca cgacgtgatc
 901 ggcaacatcc gccaggccca ctgcaacatc agcaccgacc gctggaacaa gacctgcag
 961 caggtgatga agaagctggg cgagcacttc cccaacaaga ccatccagtt caagccccac
 1021 gccggcggcg acctggagat caccatgcac agcttcaact gccgcggcga gttctctac
 1081 tgcaaacacca gcaacctgtt caacagcacc taccacagca acaacggcac ctacaagtac
 1141 aacggcaaca gcagcagccc catcacctg cagtgaaga tcaagcagat cgtgcgcatg
 1201 tggcagggcg tgggcccaggc cacctacgcc ccccccacg ccggcaacat cacctgccgc
 1261 agcaacatca ccggcatcct gctgaccgc gacggcggct tcaacaccac caacaacacc
 1321 gagaccttcc gcccggcgcc cggcgacatg cgcgacaact ggcgcagcga gctgtacaag
 1381 tacaaggtgg tggagatcaa gcccctgggc atcgcccca ccaaggccaa gagcagcgtg
 1441 gtgcagagcg agaagagcgc cgtgggcac ggcgccgtgt tcctgggctt cctgggcgcc
 1501 gccggcagca ccatgggcgc cggcagcatc accctgaccg tgcaggccc ccagctgctg
 1561 agcggcatcg tgcagcagca gagcaacctg ctgaaggcca tcgaggccca gcagcacatg
 1621 ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcgtgctggc catcagcgc
 1681 tacctgaagg accagcagct gctgggcac tggggctgca gcggccgct gatctgcacc
 1741 accgccgtgc cctggaacag cagctggagc aacaagagcg agaaggacat ctgggacaac
 1801 atgacctgga tgcagtggga ccgcgagatc agcaactaca ccggcctgat ctacaacctg
 1861 ctggaggaca gccagaacca gcaggagaag aacgagaagg acctgctgga gctggacaag
 1921 tggacaaccc tgtggaactg gttcgacatc agcaactggc cctgtacat ctaa

FIGURE 108 (SEQ ID NO:187)

gp140.mod.TV1.GM161

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgacct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaaca caccaacggc accggcatct acaacatcga ggagatgaag
481 cagtgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacgccctg
541 ttctaccgcc tggacatcgt gcccctgaac gagaacagcg acaacttcac ctaccgctg
601 atcaactgca acaccgacac catcacccag gcctgcccc aagtgagctt cgaccccatc
661 cccatccact actgcccc cgccggctac gccatcctga agtgaacaa caagaccttc
721 aacggcaccg gcccctgcta caactgagc accgtgcagt gcaccacagg catcaagccc
781 gtggtgagca cccagctgct gctgaacggc agcctggccg aggagggcat catcatccgc
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtgc acctgaacga gagcgtggag
901 atcaactgca cccgccccaa caacaacacc cgcaagagcg tgcgcatcgg ccccggccag
961 gcctttctac ccaccaacga cgtgatcggc aacatccgcc aggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggga gcaattcccc
1081 aacaagacca tcagttcaa gcccacgcc ggcggcgacc tggagatcac catgcacage
1141 ttcaactgcc gcggcgagtt cttctactgc aacaccagca acctgttcaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
1261 tgcaagatca agcagatcgt gcgcatgtgg cagggcgtgg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgccgagc aacatcaccg gcatcctgct gaccgcgac
1381 ggcggcttca acaccacaa caacaccgag acctccgcc ccggcgcgcg cgacatgcgc
1441 gacaactggc gcagcgagct gtacaagtac aagtggtgg agatcaagcc cctgggcatc
1501 gccccacca aggccaagcg ccgctgttg cagcgcgaga agcgcgccgt gggcatcggc
1561 gccgtgttcc tgggttctt ggcgccgcc ggagcacca tggcgccgc cagcatcacc
1621 ctgaccgtgc agggccgcca gctgctgagc ggcatcgtgc agcagcagag caacctgctg
1681 aaggccatcg agggccagca gcacatgctg cagctgaccg ttgggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgtac ctgaaggacc agcagctgct gggcatctgg
1801 ggctgcagcg gccgcctgat ctgcaccacc gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caactgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactgggt cgacatcagc
2041 aactggccct ggtacatcta a

FIGURE 109 (SEQ ID NO:188)

gp140mod.TV1.GM161-195-204

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtgtt ggatctgggg catcctgggc
 61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
 121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
 181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
 241 gagatcgtgc tgggcaactg gaccgagaac ttcaacatgt ggaagaacga catggccgac
 301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 accccctgtg gcgtgacct gaactgcacc gacaccaacg tgaccggcaa ccgaccgtg
 421 accggcaaca gcaccaaca caccaacggc accggcatct acaacatcga ggagatgaag
 481 cagtgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacgccctg
 541 ttctaccgcc tggacatct gccctgaac gagaacagcg accagttcac ctaccgctg
 601 atcaactgcc agaccagcac catcaccag gcctgcccc aggtgagctt cgacccatc
 661 cccatccact actgcgcccc cgccggctac gccatcctga agtgcaaca caagacctt
 721 aacggcaccg gccctgcta caactgagc accgtgcagt gacccacgg catcaagccc
 781 gtgtgtgagc cccagctgct gctgaacggc agcctggcgg aggaggcat catcatccg
 841 agcgagaacc tgaccgagaa caccaagacc atcatcgtg acctgaacga gagcgtggag
 901 atcaactgca cccgccccaa caacaacac cgcaagagcg tgcgcatcgg cccggccag
 961 gccttctacg ccaccaacga cgtgatcggc aacatccgc agggccactg caacatcagc
 1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggcga gcacttccc
 1081 aacaagacca tccagttaa gcccacgcc ggcggcgacc tggagatcac catgcacagc
 1141 ttcaactgcc gcggcgagtt ctctactgc aacaccagca acctgttaa cagcacctac
 1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
 1261 tgcaagatca agcagatcgt gcgcatgtgg caggcgctgg gccaggccac ctacgcccc
 1321 cccatcgccg gcaacatcac ctgccgcagc aacatcaccg gcatcctgct gaccgcgac
 1381 ggcggttca acaccacaa caacaccgag acctccgcc ccggcgcgcg cgacatcgcc
 1441 gacaactggc gcagcgagct gtacaagtac aaggtgtgtg agatcaagcc cctgggcatc
 1501 gccccacca aggccaagcg ccgctgtgtg cagcgcgaga agcgcgccgt gggcatcggc
 1561 gccgtgttcc tgggttctt ggcgccgcc ggagcacca tggcgccgc cagcatcacc
 1621 ctgaccgtgc agggccgcca gctgctgagc ggcatcgtgc agcagcagag caacctgtg
 1681 aaggccatcg agggccagca gcacatgctg cagctgaccg tgtggggcat caagcagctg
 1741 caggcccgcg tgctggccat cgagcgctac ctgaaggacc agcagctgct gggcatctgg
 1801 ggctgcagcg gccgcctgat ctgaccacc gccgtgccct ggaacagcag ctggagcaac
 1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
 1921 aactacaccg gcctgatcta caactgtg gaggacagcc agaaccagca ggagaagaac
 1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactggtt cgacatcagc
 2041 aactggccct ggtacatcta a

FIGURE 110 (SEQ ID NO:189)

gp140mod.TV1.GM161-204

1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggatctgggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccaccctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag
241 gagatcgtgc tgggcaactg gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctgt gcgtgacct gaactgcacc gacaccaacg tgaccggcaa ccgcaccgtg
421 accggcaaca gcaccaaca caccaacggc accggcatct acaacatga ggagatgaag
481 cagtgcagct tcaacgccac caccgagctg cgcgacaaga agcacaagga gtacggccctg
541 ttctaccgcc tggacatcgt gccctgaac gagaacagcg acaacttac ctaccgcctg
601 atcaactgcc agaccagcac catcaccag gcctgcccc aggtgagctt cgacccatc
661 cccatccact actgcgcccc cgcgggtac gccatcctga agtgaacaa caagacctc
721 aacggcaccc gccctgcta caactgagc accgtgcagt gcaccacgg catcaagccc
781 gtggtgagca cccagctgct gctgaacggc agcctggccg aggagggcat catcatccg
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtg acctgaacga gagcgtggag
901 atcaactgca cccgccccaa caacaacacc cgcaagagcg tgcgcatcgg ccccgccag
961 gcctcttacg ccaccaacga cgtgatcggc aacatccgc aggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctgggcca gcacttccc
1081 aacaagacca tccagtcaa gcccacgcc ggcgccgacc tggagatcac catgcacagc
1141 ttcaactgcc gcggcgagt ctctactgc aacaccagca acctgttaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
1261 tgcaagatca agcagatcgt gcgcatgtgg caggcgctgg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgccgagc aacatcaccc gcacccctgt gaccgagc
1381 ggcggttca acaccacaa caacaccgag acctccgcc ccggcgccgg cgacatgcgc
1441 gacaactggc gcagcgagct gtacaagtac aagggtgtgg agatcaagcc cctgggcac
1501 gccccacca agccaagcg ccgctgtgtg cagcgcgaga agcgccgtt gggcatcggc
1561 gccgtgttcc tgggttccct gggcgccgc ggagcacca tggcgccgc cagcatcacc
1621 ctgaccgtgc aggcccgcca gctgctgagc ggcatcgtgc agcagcagag caacctgctg
1681 aaggccatcg aggcccgca gcacatgctg cagctgaccg tgtggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgctac ctgaaggacc agcagctgct gggcatctgg
1801 ggctgcagcg gccgcctgat ctgaccacc gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggatgc agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caacctgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactggtt cgacatcagc
2041 aactggccct ggtacatcta a

FIGURE 111 (SEQ ID NO:190)

gp140mod.TV1.GM-V1V2

```
1 atgcgcgtga tgggcaccca gaagaactgc cagcagtggg ggaatctggg catcctgggc
61 ttctggatgc tgatgatctg caacaccgag gacctgtggg tgaccgtgta ctacggcgtg
121 cccgtgtggc gcgacgcaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
181 accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgccc caacccccag
241 gagatcgtgc tgggcaacgt gaccgagaac ttcaacatgt ggaagaacga catggccgac
301 cagatgcacg aggacgtgat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
361 acccccctg gcgtgacct gcagtgcacc gacaccagg tgaccggcca gcgcaccgtg
421 accggccaga gacccagaa caccagggc accggcatct acaacatga ggagatgaag
481 cagtgcagct tccaggccac caccagctg cgcgacaaga agcacaagga gtacgccctg
541 ttctaccgcc tggacatct gcccctgaac gagaacagcg accagttcac ctaccgcctg
601 atcaactgcc agaccagcac catcaccag gcctgcccc aggtgagctt cgaccccatc
661 cccatccact actgcgccc cgccggctac gccatcctga agtgcaaaa caagaccttc
721 aacggcaccc gcccctgcta caacgtgagc accgtgcagt gacccacgg catcaagccc
781 gtggtgagca ccagctgct gctgaacggc agcctggccg aggaggcat catcatccgc
841 agcgagaacc tgaccgagaa caccaagacc atcatcgtc acctgaacga gagcgtggag
901 atcaactgca ccgccccaa caacaacacc cgcaagagcg tgcgcatcg ccccgccag
961 gccttctacg ccaccaacga cgtgatcggc aacatccgc agggccactg caacatcagc
1021 accgaccgct ggaacaagac cctgcagcag gtgatgaaga agctggcgca gcacttcccc
1081 aacaagacca tccagttaa gcccacgcc ggcggcgacc tggagatcac catgcacagc
1141 ttcaactgcc gcggcgagt ctctactgc aacaccagca acctgtcaa cagcacctac
1201 cacagcaaca acggcaccta caagtacaac ggcaacagca gcagcccat caccctgcag
1261 tgcaagatca agcagatct gcgcatgtg cagggcgtgg gccaggccac ctacgcccc
1321 cccatcgccg gcaacatcac ctgccgcagc aacatcacc gcacatctgt gacccgcgac
1381 ggcggttca acaccacaa caacaccgag acctccgcc ccggcgccgg cgacatgcg
1441 gacaactggc gcagcgagct gtacaagtac aagggtgtgg agatcaagcc cctgggcatc
1501 gccccacca aggccaagcg ccgctgtgtg cagcgcgaga agcgcgccgt gggcatcggc
1561 gccgtgttcc tgggttctt gggcgccgcc ggagcacca tgggcgccgc cagcatcacc
1621 ctgaccgtgc agggccgcca gctgctgagc ggcatcgtgc agcagcagag caacctgctg
1681 aaggccatcg agggccagca gcacatgctg cagctgaccg tgtggggcat caagcagctg
1741 caggcccgcg tgctggccat cgagcgtac ctgaaggacc agcagctgct ggcatctg
1801 ggctgcagcg gccgcctgat ctgcaccac gccgtgccct ggaacagcag ctggagcaac
1861 aagagcgaga aggacatctg ggacaacatg acctggtatg agtgggaccg cgagatcagc
1921 aactacaccg gcctgatcta caacctgctg gaggacagcc agaaccagca ggagaagaac
1981 gagaaggacc tgctggagct ggacaagtgg aacaacctgt ggaactggtt cgacatcagc
2041 aactggccct ggtacatcta a
```

FIGURE 112 (SEQ ID NO: 191)

gp140modC8.2mut7.delV2.Kozmod.Ta

1 gccacatgc gcgtgatggg caccagaag aactgccagc agtgggtgat ctggggcatc
61 ctgggcttct ggatgctgat gatctgaac accgaggacc tgtgggtgac cgtgtactac
121 ggcgtgcccg tgtggcgcga cgccaagacc accctgttct gcgccagcga cgccaaggcc
181 tacgagaccg aggtgcacaa cgtgtgggcc acccacgcct gcgtgcccac cgacccaac
241 cccagagaga tcgtgctggg caactgacc gagaactca acatgtgga gaacgacatg
301 gccgaccaga tgcacgagga cgtgatcagc ctgtgggacc agagcctgaa gccctgcgtg
361 aagctgacc ccctgtgcgt gacctgaac tgcaccgaca ccaactgac cggcaaccgc
421 accgtgaccg gcaacagcac caacaacacc aacggcaccg gcatctacaa catcgaggag
481 atgaagaact gcagcttcaa cgccggcgcc ggccgcctga tcaactgaa caccagcacc
541 atcaccagc cctgcccac ggtgagcttc gacccatcc ccatccacta ctgcgcccc
601 gccggctacg ccactcctgaa gtgcaacaac aagacctta acggcaccgg cccctgctac
661 aacgtgagca ccgtgcagtg caccacggc atcaagccc tggtagcac ccagtgtctg
721 ctgaacgga gcctggccga ggaggcatc atcatcgca gcgagaacct gaccgagaac
781 accaagacca tcatctgca cctgaacgag agcgtggaga tcaactgac ccgcccac
841 aacaacacc gcaagagcgt gcgatcggc cccggccagg ccttctacg caccaacgac
901 gtgatcgga acatccgca ggccactgc aacatcagca ccgaccgtg gaacaagacc
961 ctgcagcagg tgatgaagaa cctggcgag cacttccca acaagacct ccagttcaag
1021 cccacgccc gcggcgacct ggagatcacc atgcacagct tcaactgcc cgcgagttc
1081 ttctactga acaccagca cctgttaac agcacctacc acagcaaca cggcacctac
1141 aagtacaac gcaacagcag cagcccatc accctgcagt gcaagatcaa gcagatcgtg
1201 cgcatgtggc agggcgtggg ccaggccacc tacgcccc ccacgcccg caacatcacc
1261 tgccgagca acatcaccg catcctgctg acccgcgacg gcggcttcaa caccaccaac
1321 aacaccgaga cttccgccc cgccggcggc gacatgcgag acaactggcg cagcgagctg
1381 tacaagtaca aggtggtgga gatcaagccc ctgggcatcg ccccccacaa ggccatcagc
1441 agcgtggtgc agagcgagaa gagcgcgtg ggcatcggcg ccgtgttct gggcttctg
1501 ggcgccccc gcagcaccat gggcgccgc agcatcacc tgaccgtgca ggcccgcag
1561 ctgctgagcg gcatcgtgca gcagcagagc aacctgtga aggccatga ggcccagcag
1621 cacatgtgc agctgaccgt gtggggcatc aagcagctg agggccgct gctggccatc
1681 gagcgctacc tgaaggacca gcagctgctg ggcatctggg gctgcagcgg ccgctgatc
1741 tgcaccacc ccgtgccctg gaacagcagc tggagcaaca agagcgagaa ggacatctg
1801 gacaacatga cctggatgca gtgggaccgc gagatcagca actacaccgg cctgatctac
1861 aacctgctgg aggacagca gaaccagcag gagaagaac agaaggacct gctggagctg
1921 gacaagtga acaacctgtg gaactggtc gacatcagca actggccctg gtacatctaa
1981 a

Translation of:		451		500
gp140mod.TV1.delV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQREKRAVGIGAVFLGFLGA		
gp140mod.TV1.mut1.dV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQREKSAVGIGAVFLGFLGA		
gp140mod.TV1.mut2.dV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQSEKSAVGIGAVFLGFLGA		
gp140mod.TV1.mut3.dV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQSEKSAVGIGAVFLGFLGA		
gp140mod.TV1.mut4.dV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQSEKSAVGIGAVFLGFLGA		
gp140mod.TV1.mut7.delV2	(451)	RDNRSELYKYKVVVEIKPLGIAPTAKRRVVQSEKSAVGIGAVFLGFLGA		

FIGURE 113

Translation of:			
gp140mod.TV1	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	150
gp140mod.TV1.GM161	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	
gp140mod.TV1.GM161-204	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	
gp140mod.TV1.GM161-195-204	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	
gp140mod.TV1.GM-V1V2	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	
Consensus	(101)	QMHEDVISLWDQSLKPCVKLTPLCVTLNCTDTNVTGNRTVTVTGNSTNNNTNG	
Translation of:			
gp140mod.TV1	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	200
gp140mod.TV1.GM161	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	
gp140mod.TV1.GM161-204	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	
gp140mod.TV1.GM161-195-204	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	
gp140mod.TV1.GM-V1V2	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	
Consensus	(151)	TGIYNIIEEMKNCSEFNATTELRLDKKHKEYALFYRLDIVPLNENSDNFTYRL	
Translation of:			
gp140mod.TV1	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	250
gp140mod.TV1.GM161	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	
gp140mod.TV1.GM161-204	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	
gp140mod.TV1.GM161-195-204	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	
gp140mod.TV1.GM-V1V2	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	
Consensus	(201)	INCNTSTITQACPKVSFDPIPIHYCAPAGYAILKCNKNTFNGTGPCYNVS	

FIGURE 114

FIGURE 115 (SEQ ID NO:203)

Nef-myrD124LLAA

ATGGCCGGCAAGTGGAGCAAGAGCAGCATCGTGGGCTGGCCCGCCGTGCGCGAGCG
CATCCGCCGCACCGAGCCCGCCGCCGAGGGCGTGGGCGCCGCCAGCCAGGACCTGG
ACAAGCACGGCGCCCTGACCAGCAGCAACACCGCCGCCAACAACGCCGACTGCGCC
TGGCTGGAGGCCCAGGAGGAGGAGGAGGAGGTGGGCTTCCCCGTGCGCCCCCAGGT
GCCCCTGCGCCCCATGACCTACAAGGCCGCCTTCGACCTGAGCTTCTTCCTGAAGGA
GAAGGGCGGCCTGGAGGGCCTGATCTACAGCAAGAAGCGCCAGGAGATCCTGGACC
TGTGGGTGTACCACACCCAGGGCTTCTTCCCCGGCTGGCAGAACTACACCCCCGGCC
CCGGCGTGCGCTACCCCTGACCTTCGGCTGGTGCTTCAAGCTGGTGCCCGTGGACC
CCGCGAGGTGGAGGAGGCCAACAAGGGCGAGAACAACTGCgcGgcGCACCCCATGA
GCCAGCACGGCATGGAGGACGAGGACCGCGAGGTGCTGAAGTGGAAGTTCGACAG
CAGCCTGGCCCGCCGCCACATGGCCCGCGAGCTGCACCCCGAGTACTACAAGGACT
GCGCCTAA

FIGURE 116 (SEQ ID NO:204)

Nef-myrD124LLAA

MaGKWSKSSIVGWPAVRERIRRTEPAAEGVGAASQDLDKHGALTSSNTAANNADCA
WLEAQEEEEEVGFPVRPQVPLRPMTYKAAFDLSFFLKEKGGLEGLIYSKKRQEILD
WVYHTQGFFPgWQNYTPGPGVRYPLTFGWCFKLVPVDPREVEEANKGENNCaaHPM
SQHGMEDEDREVLKWKFDSSLARRHMARELHPEYYKDCA

FIGURE 117 (SEQ ID NO:205)

gp160mod.TV2

1 atgcgcgcc gcggcatcct gaagaactac cgccactggt ggatctgggg catcctgggc
 61 ttctggatgc tgatgatgt caacgtgaag ggctgtggg tgaccgtga ctacggcgtg
 121 cccgtgggcc gcgaggccaa gaccacctg ttctgcgcca gcgacgcaa ggcctacgag
 181 aaggaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgccc caacccccag
 241 gaggtgatcc tgggcaacgt gaccgagaac ttaacatgt ggaagaacga catggtggac
 301 cagatgcagg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg
 361 acccccctgt gcgtgacct gaactgcacc aacgccaccg tgaactaaa caacaccagc
 421 aaggacatga agaactgcag cttctacgtg accaccgagc tgcgcgacaa gaagaagaag
 481 gagaacgccc tgttctaccg cctggacatc gtgcccctga acaaccgcaa gaacggcaac
 541 atcaacaact accgcctgat caactgcaac accagcgcca tcaccaggc ctgcccgaag
 601 gtgagcttcg accccatccc catccactac tgcgccccg ccggctacgc cccctgaag
 661 tgcaacaaca agaagttaaa cgcatcggc cctgcgaca acgtgagcac cgtgcagtgc
 721 acccacggca tcaagcccg ggtgagcacc cagctgtgc tgaacggcag cctggccgag
 781 gaggagatca tcatccgag cgagaacctg accaacaacg tgaagacat catcgtgcac
 841 ctgaacgaga gcatcgagat caagtgcacc cgccccggca acaacaccg caagagcgtg
 901 cgcacggccc ccggccaggc cttctacgcc accggcgaca tcatcgcca catccgccag
 961 gccactgca acatcagcaa gaacgagtgg aacaccacc tgcagcgct gagccagaag
 1021 ctgcaggagc tgttcccaa cagcaccggc atcaagttc cccccacag cggcggcgac
 1081 ctggagatca cccccacag cttcaactgc ggcgcgagt tcttctactg caacaccacc
 1141 gacctgtta acagaccta cagcaacggc acctgcacca acggcacctg catgagcaac
 1201 aacaccgagc gcatcaccct gcagtgcgc atcaagcaga tcatcaacat gtggcaggag
 1261 gtggcgccgc ccatgtacgc ccccccatc gccggcaaca tcacctgccg cagcaacatc
 1321 accggcctgc tctgacctg cgacggcggc gacaacaaca ccgagaccga gaccticcgc
 1381 cccggcgccg gcgacatgc cgacaactgg cgcagcgagc tgtacaagta caaggtggtg
 1441 gagatcaagc cctgggcgt ggccccacc gccccaagc gccgcgtggt ggagcgcgag
 1501 aagcgcgccg tgggcatcgg cgccgtgtc ctgggttcc tgggcgccgc cggcagcacc
 1561 atggcgccg ccagcatcac cctgaccgtg caggcccgcc agctgctgag cggcatcgtg
 1621 cagcagcaga gcaacctgct gcgcgccatc gagggccagc agcacatgct gcagctgacc
 1681 gtgtggggca tcaagcagct gcaggccgc gtgctggcca tcgagcgcta cctgcaggac
 1741 cagcagctgc tgggcctgtg gggctgcagc ggcaagctga tctgcaccac caactgctg
 1801 tggaaacaga gctggagcaa caagaccag agcgacatct gggacaacat gacctggatg
 1861 cagtgggacc gcgagatcag caactacacc aacacatct accgcctgct ggaggacagc
 1921 cagagccagc aggagcgcaa cgagaaggac ctgctggccc tggaccgtg gaacaacctg
 1981 tggáactggt tcagcatcac caactggctg tggatcatca agatcttcat catgatcgtg
 2041 ggcgccctga tcggcctgc catcatctc gccgtgctga gcctggtgaa ccgcgtgcgc
 2101 cagggtaca gccccctgag cctgcagacc ctgatccca acccccgccg ccccgaccgc
 2161 ctggcgccga tcgaggagga gggcgccgag caggacagca gccgcagcat ccgcctggtg
 2221 agcggttcc tgacctggc ctgggacgac ctgcgcagcc tgtgcctgtt ctgctaccac
 2281 cgcctgcgcg acttcatcct gatcgtggtg cgcgccgtgg agctgctggg ccacagcagc
 2341 ctgcgcggcc tgcagcgccg ctggggcacc ctgaagtacc tgggcagcct ggtgcagtac
 2401 tggggcctgg agctgaagaa gagcgccatc aacctgtgg acaccatgc catcgccgtg
 2461 gccgaggcca ccgaccgcat cctggagttc atccagaacc tgtccgcgg catccgcaac
 2521 gtgccccgcc gcatccgcca gggcttcgag gccgccctgc agtaa

Figure 118
(Sheet 1 of 1)

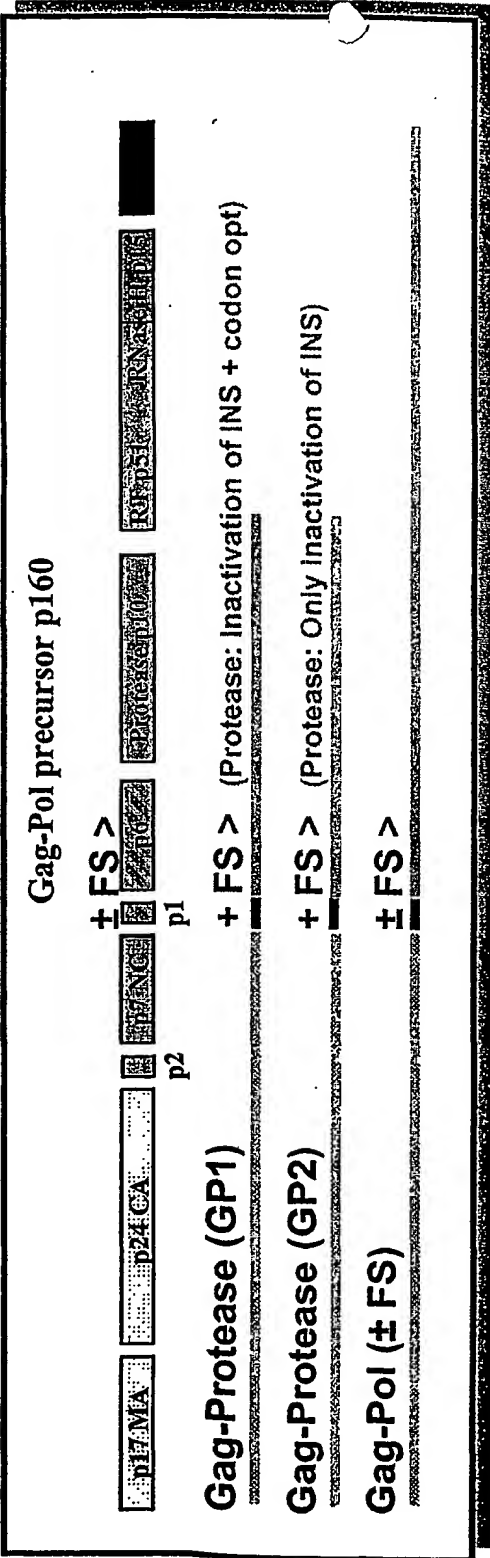
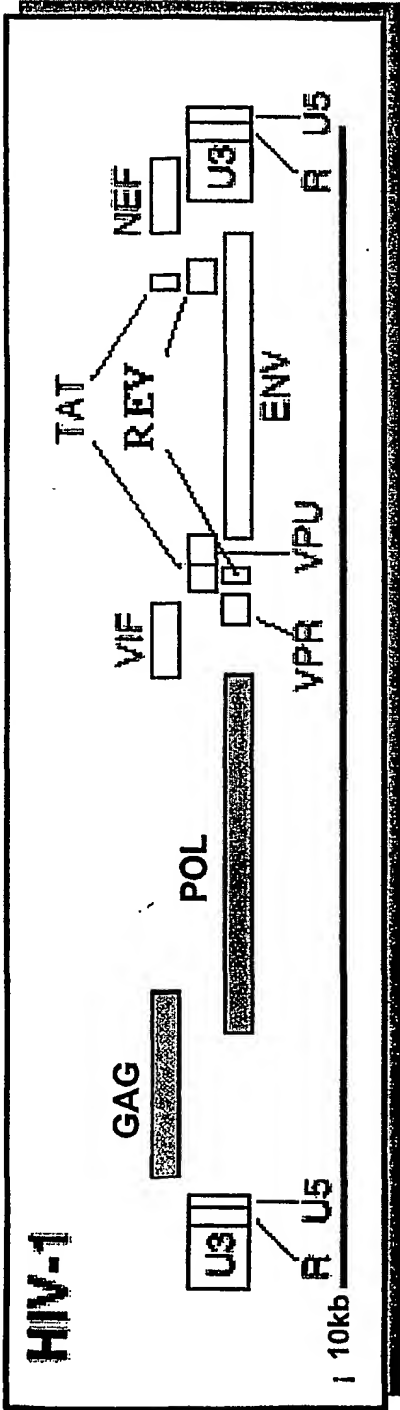


Figure 119

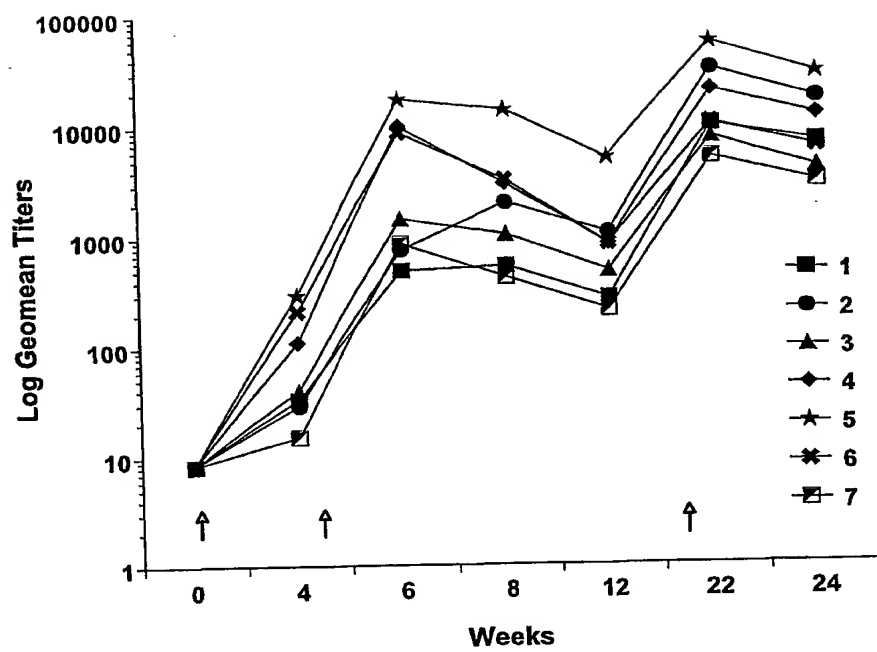


Figure 120

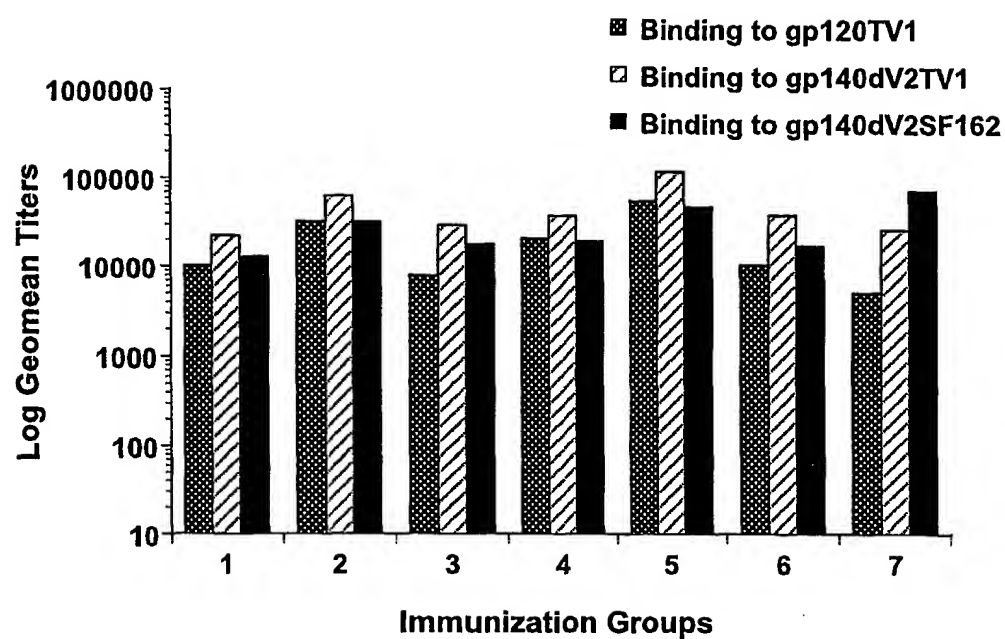


Figure 121

Group	Animal	% Virus Inhibition			
		Post-2 nd DNA (1:20)	Post-2 nd - DNA (1:100)	Post-Prot (1:100)	Post-Prot (1:500)
1	1	0	60	0	17
	2	34	59	50	21
	3	0	0	12	38
	4	95	92	83	57
2	5	100	69	99	99
	6	0	28	27	35
	7	0	0	43	0
	8	95	38	79	74
3	9	40	0	61	26
	10	0	0	0	0
	11	94	41	91	57
	12	0	0	12	19
4	13	100	86	78	18
	14	20	0	68	0
	15	99	70	100	31
	16	0	33	0	24
5	17	100	67	100	75
	18	69	36	100	53
	19	58	33	NA	NA
	20	99	80	92	39
6	21	NA	NA	NA	NA
	22	78	12	100	88
	23	67	63	92	17
	24	70	62	77	0
7	29	100	100	74	68
	30	81	63	55	28
	31	100	79	100	91
	32	100	78	100	45
Sub B positive serum	20480	100	100	100	100

Figure 122

Group	Animal	% Virus Inhibition		ELISA Titer
		TV1	TV2	
1	1	0	38	19716
	2	25	67	37994
	3	0	0	7529
	4	0	79	41963
2	5	30	51 [#]	112768
	6	0	0	57677
	7	23	9	26247
	8	47	78	90376
3	9	0	42	62004
	10	13	0	5741
	11	0	36 [#]	53599
	12	21	12	37597
4	13	0	22 [#]	45543
	14	0	0	24885
	15	0	17 [#]	87556
	16	28 [#]	59	19838
5	17	72	80	124618
	18	0	77	143905
	19	NA	NA	NA
	20	19	56 [#]	91808
6	21	NA	NA	NA
	22	34	44	31413
	23	51	50 [#]	62925
	24	22	31 [#]	28620
	29	0	9	62604
	30	0	50 [#]	15932
	31	0	58	22418
	32	41	0	21119
Sub B positive pool		46	56	NA
Sub C positive pool		36	85	NA

Figure 123

Group	Animal	% Virus Inhibition			ELISA titer
		TV1	Du174	SF162	
1	1	28	20	12	19716
	2	33	19	9	37994
	3	0	0	0	7529
	4	52	61	79	41963
2	5	33	0	95	112768
	6	3	0	14	57677
	7	0	0	0	26247
	8	54	0	86	90376
3	9	0	52	73	62004
	10	0	58	15	5741
	11	0	0	71	53599
	12	0	0	0	37597
4	13	15	0	69	45543
	14	0	0	0	24885
	15	0	13	0	87556
	16	14	0	0	19838
5	17	0	0	0	124618
	18	0	0	30	143905
	19	NA	NA	NA	NA
	20	63	0	56	91808
6	21	NA	NA	NA	NA
	22	24	NV	38	31413
	23	7	65	76	62925
	24	0	NV	NV	28620
7	29	32	0	82	62604
	30	6	NV	0	15932
	31	0	0	98	22418
	32	34	0	0	21119

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